FORMULATION AND EVALUATION OF POLYMERIC NANOPARTICLES OF FELODIPINE

Dissertation submitted to

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DEPARTMENT OF PHARMACEUTICS

COLLEGE OF PHARMACY

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MADURAI – 625020

APRIL – 2014

CERTIFICATES

بسم الله الرحمن الرحيم
In the Name of Allāh, the Most Gracious, the Most Merciful

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CERTIFICATE

This is to certify that the dissertation entitled**, "Formulation and Evaluation of Polymeric Nanoparticles of Felodipine"** submitted by **Mr. A. Manikkavasagan, (M.Pharm II year)**, in partial fulfillment of the requirement for the Degree of **Master of Pharmacy** in **Pharmaceutics**, is a bonafide work carried out by him, under my guidance and supervision in the Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai,during the academic year 2013 – 2014.

This dissertation is forwarded to the Controller of Examinations, The Tamilnadu Dr. M.G.R. Medical University, Chennai-32.

Place : Madurai

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INTRODUCTION

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INTRODUCTION

Nanotechnology:

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale (one-billionth of a meter). In the past few years nanotechnology has grown by leaps and bounds, and this multidisciplinary scientific field is undergoing explosive development . It can prove to be a boon for human health care, because nano science and nanotechnologies have a huge potential to bring benefits in areas as diverse as drug development, water decontamination, information and communication technologies, and the production of stronger, lighter materials. Human health-care nanotechnology research can definitely result in immense health benefits. The genesis of nanotechnology can be traced to the promise of revolutionary advances across medicine, communications, genomics, and robotics. A complete list of the potential applications of nanotechnology is too vast and diverse to discuss in detail, but without doubt, one of the greatest values of nanotechnology will be in the development of new and effective medical treatments. This review focuses on the potential of nanotechnology in medicine, including the development of nanoparticles for drug and gene delivery and diagnostics. These technologies will extend the limits of current molecular diagnostics and permit accurate diagnosis as well as the development of personalized medicine.

The prefix “nano” derives from the Greek word for “dwarf”. One nanometer (nm) is equal to one-billionth of a meter, or about the width of 6 carbon atoms or 10 water molecules. (Sahoo S K et al., 2007).

Types:

i. **Nanoscience:**

Nanoscience refers to scientific activity that occurs with in the range of 1-100 nanometers and is usually aimed at constructing devices with atomic precision. (Christine M shea et al., 2005).

ii. **Nanomedicine:**

Nanomedicine defined as the monitoring, repair, construction, and control of human biological systems at the molecular level, using engineered nano devices and nano structures. It can also be regarded as another implementation of nanotechnology in the field of medical sciences and diagnostics. One of the most important issues is the proper distribution of drugs and other therapeuctic agents within the patient's body.

(Sahoo S K et al., 2007).

iii. **Nanotechnology:**

Nanotechnology is the application of nanoscientific developments toward some commercial objective. It is enabled by advances in and the convergence of the fields of physics, chemistry, biology, materials science, and engineering principles and tools, and encompasses the entire domain of the building, integration and application of nanoscale structures in to larger material components, systems and architectures.

(Christine M shea et al., 2005).

Nanotechnology in drug delivery:

The development of delivery systems for small molecules, proteins and DNA has been impacted to an enormous degree over the past decade by nanotechnology, and has led to the development of entirely new and somewhat unpredicted fields. For

the pharmaceutical industry, novel drug delivery technologies represent a strategic tool for expanding drug markets. The technology can address issues associated with current pharmaceuticals such as extending product life (line extension), or can add to their performance and acceptability, either by increasing efficacy or improving safety and patient compliance. In addition, the newer drugs developed with the help of computational chemistry using the knowledge gained from the human genome project require drug delivery systems for their effective use. This technology permits the delivery of drugs that are highly water-insoluble or unstable in the biological environment. It is expected that novel drug delivery systems can make a significant contribution to global pharmaceutical sales. This is illustrated by the fact that approximately 13% of the current global pharmaceutical market is accounted for by sales of products incorporating a drug delivery system. In recent years, many new pharmaceutical companies have been established that can provide expertise in innovative delivery technology. Also, many established pharmaceutical industries are gearing-up their efforts towards developing more effective and performance-based new drug delivery systems. The demand for drug delivery systems in the United States alone is expected to grow nearly 9% annually to more than US$82 billion by 2007. (Sanjeeb K Sahoo et al., 2003).

Significance of Drug Delivery and Targeting:

Although opportunities to develop nanotechnology-based efficient drug delivery systems extend into all therapeutic classes of pharmaceuticals, the development of effective treatment modalities for the respiratory, central nervous system and cardiovascular disorders remains a financially and therapeutically significant need. Many therapeutic agents have not been successful because of their limited ability to reach to the target tissue. In addition, the faster growth opportunities

are expected in developing delivery systems for anti-cancer agents, hormones and vaccines because of safety and efficacy shortcomings in their conventional administration modalities. For example, in cancer chemotherapy, cytostatic drugs damage both malignant and normal cells alike. Thus, a drug delivery strategy that selectively targets the malignant tumor is very much needed. Additional problems include drug instability in the biological milieu and premature drug loss through rapid clearance and metabolism. Similarly, high protein binding of certain drugs such as protease inhibitors limits their diffusion to the brain and other organs. However, nanotechnology for drug delivery applications may not be suitable for all drugs, especially those drugs that are less potent because the higher dose of the drug would make the drug delivery system much larger, which would be difficult to administer. (Sanjeeb K Sahoo et al., 2003).

Types of collodal nanocarriers

1. Polymeric nanoparticles:

Polymeric nanoparticles (PNPs) are prepared from a synthetic polymeric block to increase the circulation half-life and to reduce phagocytic uptake and inactivation of the therapeutic moiety and can be used to deliver and target therapeutic agents. They are formulated by incorporation of biodegradable polymers to maximize tissue compatibility and minimize cytotoxicity. Polymers approved by the U.S. Food and Drug Administration (FDA) for administration in human beings are polylactic acid (PLA), poly(glycolic acid) (PGA), PLGA, poly-ecaprolactone, and poly(methyl methacrylate). For example, PLA and PLGA can easily be hydrolyzed into individual monomers (lactic acid or glycolic acid), which are removed from the body via normal metabolic pathways.

Methods of preparation of PNPs fall into two major classes:

One deals with the polymerization of monomers (eg, emulsion and dispersion polymerization), whereas the other essentially involves dispersion of polymers (eg, salting out, emulsification- diffusion, and nanoprecipitation). Reports show that higher entrapment efficiency in polymeric nanoparticles can be achieved by incorporation of drug during their preparation rather than adsorption on preformed nanoparticles. Drug release takes place through their simultaneous biodegradation, followed by desorption, diffusion, or erosion. (Mishra B et al., 2010).

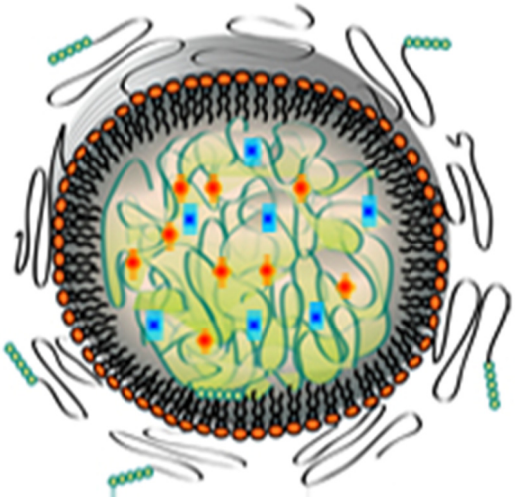


FIGURE 1: STRUCTURE OF POLYMERIC NANOPARTICLES

Nanospheres

Nasnospheres have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed onto their surfaces or encapsulated within the particles.

Nanocapsules

Nanocapsules are vesicular systems in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrance. In this case the active substance is usually dissolved in the inner core, but may also be adsorbed to the capsule surface. (Natarajan Jawahar et al. 2012).

2. Solid Lipid Nanoparticles:

Solid lipid nanoparticles are a comparatively stable colloidal carrier system in which melted lipid is dispersed in an aqueous surfactant by high-pressure homogenization or microemulsification. They are generally made up of a solid hydrophobic core containing the drug dissolved or dispersed. Solid lipid nanoparticles exhibit certain potential advantages over polymeric nanoparticles. They are safely taken up by brain and exhibit the least toxicity due to the biodegradable nature of the carrier lipid. Smaller size (around 10 to 200 nm) and narrow size range (100 to 200 nm) allows them to cross tight endothelial cells of the blood-brain barrier (BBB), escape from the reticuloendothelial system (RES), and bypass liver. They have comparatively higher drug entrapment efficiency, render the drug more stable in their lipid matrix, and provide a controlled release lasting up to several weeks. Their production can be scaled up with excellent reproducibility. Surface coating of solid lipid nanoparticles with hydrophilic polymers or surfactants, such as poly(ethylene glycol) (PEG), minimizes their uptake in liver cells and results in improved bioavailability. Stearic acid–PEG 2000 has been used for their stearic stabilization, whereas the use of complex lipids (mono-, di-, triglycerides of different chain lengths) results in an increased loading efficiency. (Mishra B et al., 2010).

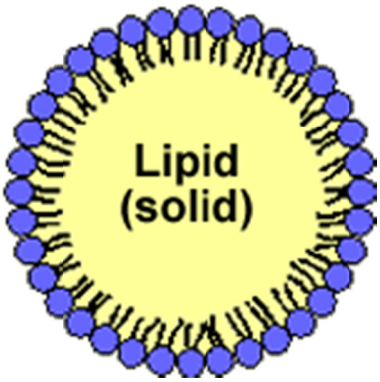


FIGURE 2: STRUCTURE OF SOLID LIPID NANOPARTICLES

3. Magnetic nanoparticles:

Magnetically targeted nanoparticulate drug delivery systems involve binding of drug with magnetic nanoparticles, such as oxidized iron (Fe) or magnetite. By virtue of their controllable sizes (ranging from 10 to 100 nm) and capacity of delivering the drug or radionucleotide in the vicinity of a target site, they provide a good scope in drug delivery. For biomedical applications, magnetic carriers must be water based, biocompatible, nontoxic, and nonimmunogenic. Various magnetic carriers, which receive external magnetic field, include nickel, cobalt, iron, and magnetite. Iron oxide is most commonly used because of its biodegradable nature, biocompatibility, super paramagnetic effects, and capacity to serve as a contrast agent in magnetic resonance imaging (MRI). Iron oxide particles are phagocytosed or endocytosed by the kupffer cell in the RES of liver, spleen, lymph, and bone marrow. Once compartmentalized within the lysosomes of RES cells, they are broken down into ferritin and/or hemosiderin, which are antiferromagnetic forms of iron. The concentration of carriers at any specific location can be manipulated by calculation of capillary flow rate, vascular permeability, and hydrodynamic condition of the individual. For therapeutic effect, magnetic nanoparticles are injected into the bloodstream, and a high gradient magnetic field is generated outside the body so as to pull them out of suspension and deliver the drug to a localized disease site. Coating with dextran or PEG improves its water dispersibility. Iron oxide magnetic nanoparticles, coated with oleic acid, were stabilized by pluronic F-127 to form a stable, water-dispersible system. (Mishra B et al., 2010).

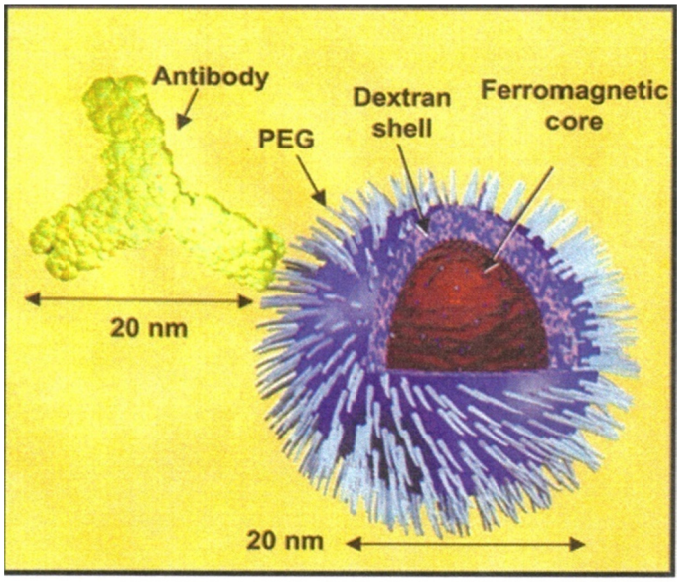


FIGURE 3: STRUCTURE OF MAGNETIC NANOPARTICLES

4. Ceramic nanoparticles:

The newly emerging area of using inorganic (ceramic) particles with entrapped biomolecules has potential applications in many frontiers of modern material science including drug delivery. Ceramic nanoparticles have several advantages such as the preparative processes are relatively similar to the well-known sol-gel process, require ambient temperature condition, and can be easily prepared with the desired size, shape and porosity. Their ultra-low size (less than 50 nm) can help them evade by the reticulo-endothelial system (RES) of the body. In addition, there are no swelling or porosity changes with change in pH. These particles effectively protect doped molecules (enzymes, drugs, etc) against denaturation induced by external pH and temperature. Such particles, including silica, alumina, titania, etc are known for their compatibility with biological systems. In addition, their surfaces can be easily modified with different functional groups. Therefore, they can be conjugated to a variety of monoclonal antibodies or ligands to target them to desired sites *in vivo*.

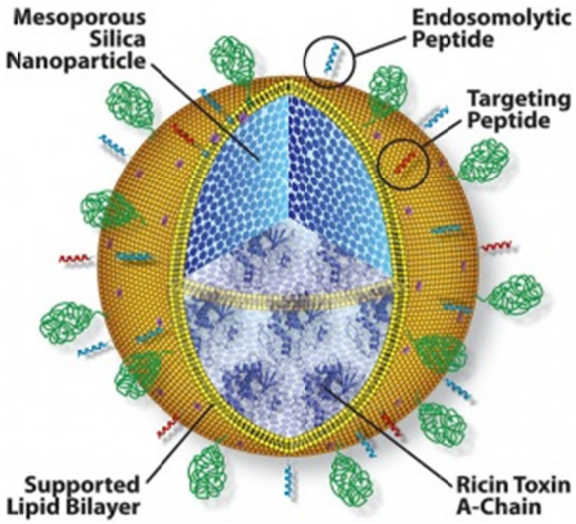


FIGURE 4: STRUCTURE OF CERAMIC NANOPARTICLES

5. Polymeric Micelles:

Polymeric micelles are nanoscopic core-shell structures created by spontaneous self-assembly of individual amphiphilic di/tri-block co-polymers, with hydrophobic core and hydrophilic surface shells or vice versa. They contain both hydrophilic and hydrophobic regions in their structure and serve as good candidates for poorly soluble drugs. Multifunctional polymeric micelles can be designed to facilitate simultaneous drug delivery and imaging. Their stability depends upon strong cohesive force between drug and core polymer segments as well as cross-linking of the shell or core, which is performed by radical polymerization. Prolonged circulation and targeted delivery of polymeric micelles is possible by designing of environment-responsive polymeric micelles (pH, light, temperature, ultrasound, etc.). The pH at a tumor site is acidic (6.5 to 7.2) compared with that of healthy tissues. Therefore, polymeric micells with hydrazone and acetal bonds, which are labile at lower pH (pH 5.0 to 7.0) and stable at physiologic pH (7.0 to 7.4), have been employed as pH-sensitive vehicles for drug delivery.

Micellar drug delivery using ultrasonic waves (20 to 40 kHz) has been explored because of its noninvasiveness and capability of targeting the drug deeply into the tumor. Such waves increase the permeability and extravasation at the tumor site, thus enhancing the drug uptake. Drug release can be modulated by ultrasound frequency, insonation, power density, pulse length, and inter pulse intervals.

Thermoresponsive polymeric micelles exhibit changes in polymeric properties in response to small alterations in temperature. They consist of a hydrophobic polymeric inner core and temperature-sensitive polymeric outer shell, which turns hydrophilic below its lower critical solution temperature (LCST) and hydrophobic above the LCST. (Mishra B et al., 2010).

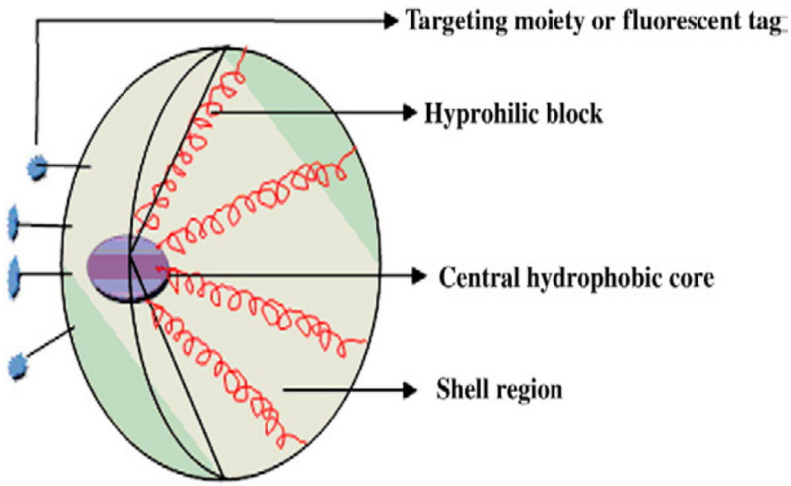


FIGURE 5: STURCTURE OF POLYMERIC MICELLES

6. Dendrimers:

The word dendrimer was derived from the greek words dendron meaning tree or branch and meros meaning part. Dendrimers can be defined as synthetic symmetrical macromolecular compounds consisting of tree-like branches around an inner core. Currently, these are emerging as effective drug delivery vehicles owing to their nanometer size range and ability to have multiple copies of surface groups for biological recognition processes. Owing to the presence of the highly branched

structure, the surfaces can be used as a suitable targeted drug delivery vehicle by conjugating with different ligands. The presence of multivalent branches with cage-like structures has been used as a suitable platform for simultaneous delivery of hydrophobic and hydrophilic drugs. (Priyambada parhi et al., 2012).

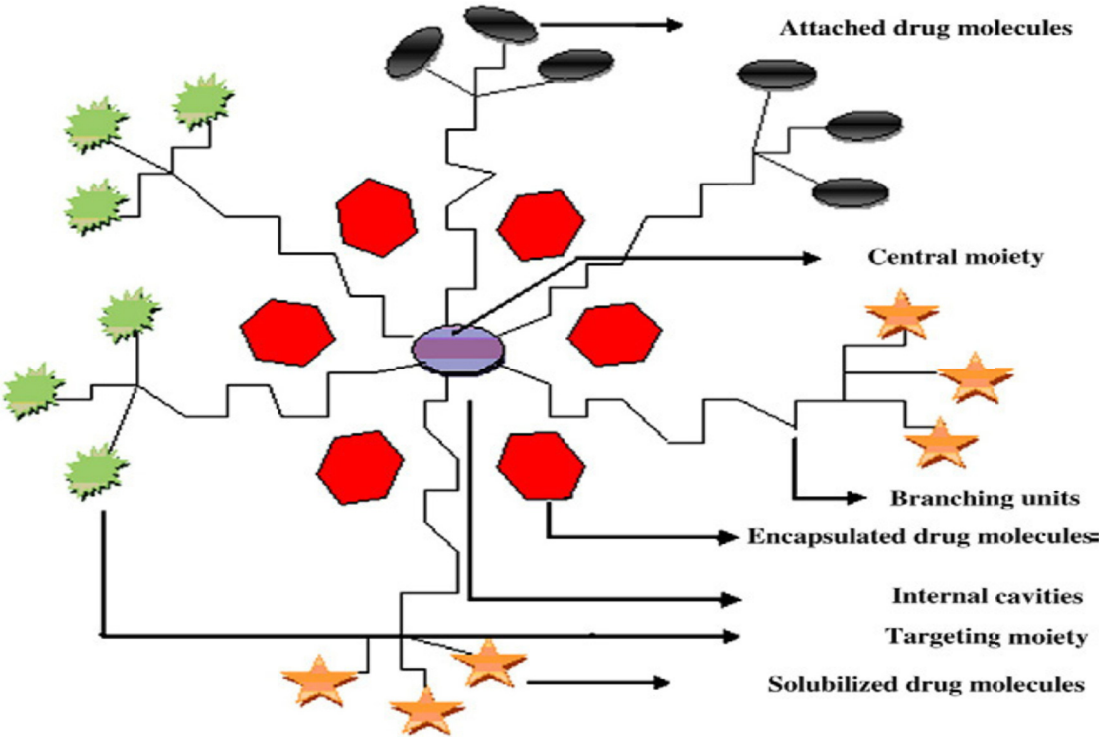


FIGURE 6: STRUCTURE OF DENDRIMERS

7. Colloidal nano liposomes:

Liposomes are small artificial vesicles of globular shape composed of aquatic pores encapsulated with amphiphilic phospholipids and cholesterol bilayer. They are widely used because of their size, both hydrophilic as well as hydrophobic character, and tissue biocompatibility. Depending on size and number of phospholipid bilayers, liposomes can be classified into small unilamellar vesicles (SUVs; single lipid layer 25 to 50 nm in diameter), large unilamellar vesicles (LUVs; heterogeneous group of vesicles), and multilamellar vesicles (MLVs; several lipid layers separated from one another by a layer of aqueous solution).

Liposomes have been investigated for the delivery of vaccine, toxoids, gene, anticancer, and anti-HIV drugs. Their blood circulation time can be increased through surface modification (eg, by attaching PEG, dextran, or poly-N-vinylpyrrolidones to the lipid bilayer). Furthermore, conjugation with targeting ligands, like monoclonal antibodies or aptamers, can enhance their tissue specificity. Liposome technology has existed for the past four decades, but they do not have enough market share due to some of their potential drawbacks, like batch-to-batch variation in manufacturing, low drug loading efficiency, and poor stability. (Mishra B et al., 2010).

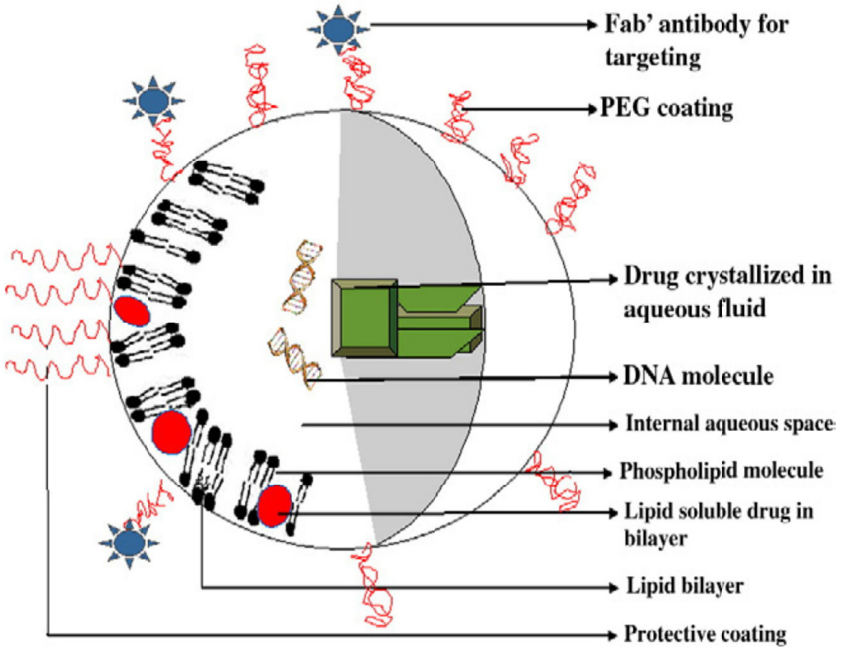


FIGURE 7: STURCTURE OF LIPOSOMES

8. Carbon nanotubes:

Carbon nanotubes are carbon cylinders composed of benzene rings and they have attracted a tremendous amount of attention regarding their use in biomedical applications. In comparison with other nano-materials, carbon nanotubes appear to be more dynamic in their biological application. Application of carbon nanotubes for the delivery of drugs to their site of action has become one of the main areas of interest for different research groups. This is mainly because of the characteristics of these

materials, including their unique chemical, physical and biological properties, their nano needle shape, hollow mono-lithic structure and their ability to obtain required functional groups on their outer layers. The shape of the carbon nanotubes enables these materials to enter the cell via different methods, such as passive diffusion across the lipid bilayer or endocytosis, whereby the carbon nanotubes attaches to the surface of the cell and is subsequently engulfed by the cell membrane. (Priyambada parhi et al., 2012).

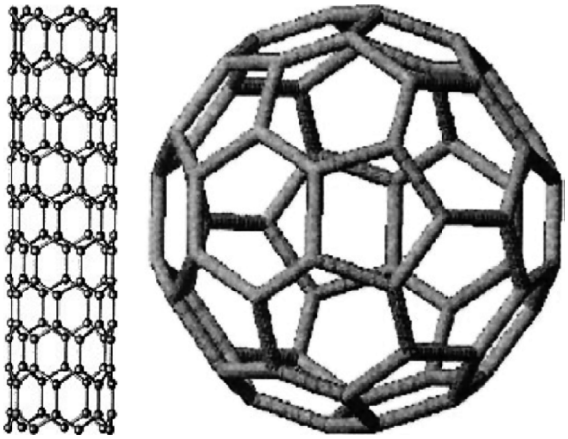


FIGURE 8: STURCTURE OF CARBON NANOTUBES

9. Polymer drug conjugates:

The advantages of polymer–drug conjugates include passive tumor targeting by enhanced permeable retention effect due to its leaky vasculature allows selective extravasation of the conjugate in tumor tissue and retained for longer period due to its poor lymphatic drainage. The other benefits of polymer-drug conjugates are reduced toxicity, overcoming multi-drug resistance, capability to remove immune stimulatory effects and enhanced solubility and bioavailability of drug conjugate in biological fluids that modulate its pharmacokinetic behaviour. The mechanism of passive tumor targeting by enhanced permeable retention effect to the tumor tissues. (Priyambada parhi et al., 2012).

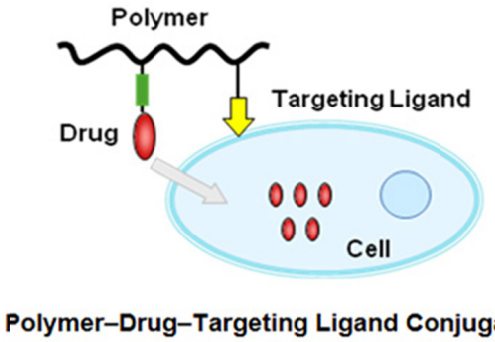


FIGURE 9: STRUCTURE OF POLYMER DRUG CONJUGATES

10. Metal and inorganic nanoparticles:

Various metals, such as gold (Au), copper (Cu), and silver (Ag), and inorganic carriers, such as silica or alumina, have been used for the preparation of nanoparticles, among which gold nanoparticles are most promptly used due to their excellent optical and photoelectric properties. Moreover, gold exhibits some specific advantages, like inertness and nontoxicity, higher stability, ease of preparation, and possibility of bioconjugation and biomodification with thiol, disulfide, and amine functional groups. Its dispersion stability can be enhanced by conjugation with thiolated PEG. Gold nanoparticles are highly effective contrast agents in cancer diagnosis and photodermal cancer therapy. Furthermore, they serve as a good vector for oligonucleotide, thiol-conjugated small interfering RNA (Si- RNA), insulin, and gene delivery. (Mishra B et al., 2010).

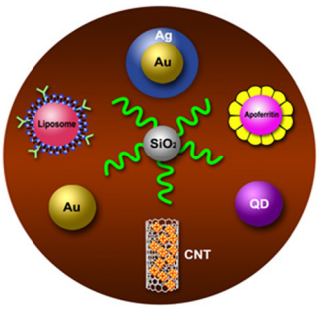


FIGURE 10: STRUCTURE OF METAL AND INORGANIC NANOPARTICLES

11. Quantum dots:

Quantum dots are colloidal semiconductor nanocrystals (up to 2 to 10 nm), composed of atoms from groups II–VI or III–Vof the periodic table, having unique optical and fluorescent properties. Those most commonly used are cadmium selenide (CdSe), cadmium telluride (CdTe), and indium arsenide (InAs). Upon their interaction with photon, they get excited and emit energy in UV, visible, or near-infrared (IR) regions, which can be detected. Owing to their small size, they can be used for the tagging of biological macromolecules, such as nucleoside and proteins. Among different elements, dihydrolipoic acid (DHLA)-coated cadmium selenide-zinc sulfide (CdSe- ZnS) QDs have shown a more stable fluorescent intensity and higher photosatbility. To enhance their water solubility and photostability, various surface modification techniques have been used. (Mishra B et al., 2010).

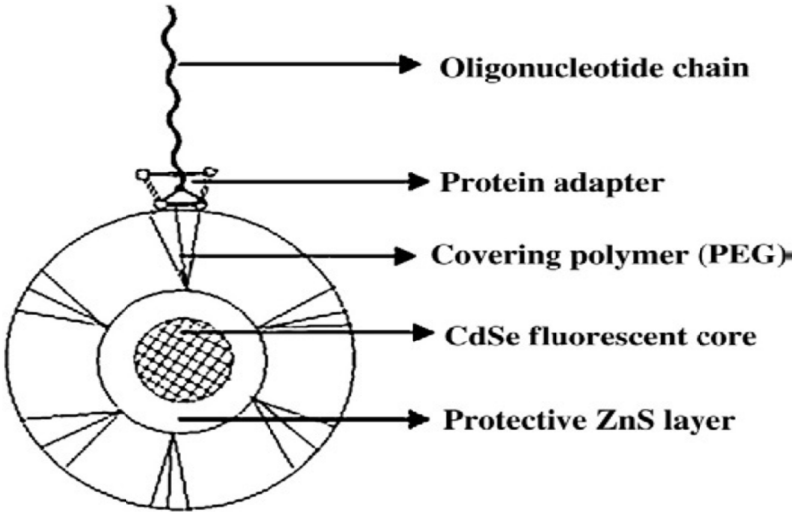


FIGURE 11: STURCTURE OF QUANTUM DOTS

12. Phospholipid micelles:

Sterically stabilized micelles (SSMs), composed of polyethylene glycol (PEGylated) phospholipids, have been introduced as safe, biocompatible nanocarriers for the delivery of poorly water-soluble drugs, especially anticancer molecules. camptothecin-containing Sterically stabilized micelles (CPT-SSMs) as a novel

nanomedicine for parenteral administration, which showed higher solubilization potential, estimable stability, and less in vitro cytotoxicity. Phospholipid based nanomicelles of indisulam were prepared by co-precipitation and reconstitution of drug and lipids. It showed 40-fold increase in drug solubility and was successfully lyophilized without addition of any lyoprotectant or cryoprotectant. (Mishra B et al., 2010).

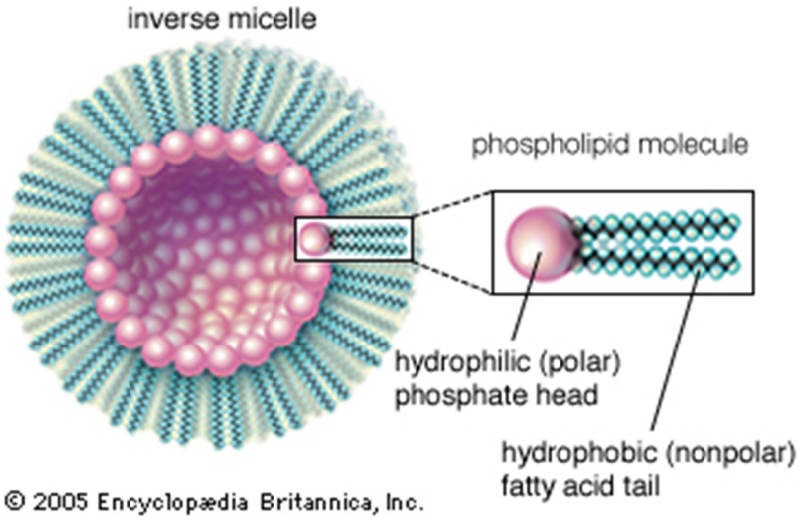


FIGURE 12: STRUCTURE OF PHOSPHOLIPID MICELLES

Applications of nanotechnology

a) Nanotechnology in drug delivery

Nanotechnologies in medicine are especially promising, and areas such as disease diagnosis, drug delivery targeted at specific sites in the body, and molecular imaging are being intensively investigated and some products undergoing clinical trials. Nanotechnology is relatively new, and although the full scope of contributions of these technological advances in the field of human health care remains unexplored, recent advances suggest that nanotechnology will have a profound impact on disease prevention, diagnosis, and treatment.

Drug solubility and cell permeability issues are also common with small molecules and biologically active compounds. Nanotechnology- based delivery systems could mitigate these problems by combining tissue- or organ-specific targeting with therapeutic action. Multifunctional nanodelivery systems could also combine targeting, diagnostic, and therapeutic actions.

The most important clinical applications of nanotechnology are likely to be in pharmaceutical development. There are already an astonishing number of emerging applications. These applications either take advantage of the unique properties of nanoparticles as drugs or components of drugs per se or are designed for new approaches to controlled release, drug targeting, and salvage of drugs with low bioavailability.

Drug bioavailability is a related problem with potential nanotechnology solutions. Nanotechnology is opening new therapeutic opportunities for many agents that cannot be used effectively as conventional oral formulations because of their poor bioavailability. In some cases, reformulation of a drug with smaller particle size may improve oral bioavailability. Nanoparticles formulations provide protection for agents

susceptible to degradation or denaturation in regions of harsh pH, and also prolong the duration of exposure of a drug by increasing retention of the formulation through bioadhesion.

Another broad application of nanotechnology is the delivery of antigens for vaccination. Mucosal immunity is extremely important in disease prevention but continues to be limited by both degradation of the vaccine and limited uptake. Recent advances in encapsulation and development of suitable animal models have demonstrated that microparticles and nanoparticles are capable of enhancing immunization.(S K Sahoo et al., 2007)

Nanobots are robots that carry out a very specific function and are just several nanometers wide. They can be used very effectively for drug delivery. Normally, drugs work through the entire body before they reach the disease affected area. Using nanotechnology, the drug can be targeted to a precise location which would make the drug much more effective and reduce the chances of possible side-effects.(Debjit bhowmik et al., 2009).

Diagnosis and Imaging:

Nanobiotech scientists have successfully produced microchips that are coated with human molecules. The chip is designed to emit an electrical impulse signal when the molecules detect signs of a disease. Special sensor nanobots can be inserted into the blood under the skin where they check blood contents and warn of any possible diseases. They can also be used to monitor the sugar level in the blood. Advantages of using such nanobots are that they are very cheap to produce and easily.(Debjit bhowmik et al., 2009).

Preventing diseases:

a. heart-attack prevention:

Nanobots can also be used to prevent heart-attacks. Heart-attacks are caused by fat deposits blocking the blood vessels. Nanobots can be made for removing these fat deposits (Harry, 2005). The following figure shows nanobots removing the yellow fat deposits on the inner side of blood vessels.

b. frying tumors:

Nanomaterials have also been investigated into treating cancer. The therapy is based on "cooking tumors" principle. Iron nanoparticles are taken as oral pills and they attach to the tumor. Then a magnetic field is applied and this causes the nanoparticles to heat up and literally cook the tumors from inside out.

c. Tissue Reconstruction:

Nanoparticles can be designed with a structure very similar to the bone structure. An ultrasound is performed on existing bone structures and then bonelike nanoparticles are created using the results of the ultrasound. The bone like nanoparticles are inserted into the body in a paste form. When they arrive at the fractured bone, they assemble themselves to form an ordered structure which later becomes part of the bone. Another key application for nanoparticles is the treatment of injured nerves. Samuel Stupp and John Kessler at Northwestern University in Chicago have made tiny rod like nano fibers called *amphiphiles*. They are capped with amino acids and are known to spur the growth of neurons and prevent scar tissue formation. Experiments have shown that rat and mice with spinal injuries recovered when treated with these nano-fibers.(Debjit bhowmik et al., 2009).

Nanotechnology in gene delivery:

nanotechnological tools in human gene therapy has been reviewed widely by davis, who described nonviral vectors based on nanoparticles (usually 50-500 nm in size) that were already tested to transport plasmid DNA. He emphasized that nanotechnology in gene therapy would be applied to replace the currently used viral vectors by potentially less immunogenic nanosize gene carriers. So delivery of repaired genes or the replacement of incorrect genes are fields in which nanoscale objects could be introduced successfully.

Nanotechnology in dental care:

Nanotechnology will have future medical applications in the field of nanodentistry. Nanodentistry will make it possible to maintain near-perfect oral health through the use of nanomaterials, biotechnology, and nanorobotics.

A colloidal suspension containing millions of active analgesic dental nanorobotic particles could be instilled on the patient's gingivae. These nanorobots, after contacting the surface of the crown or mucosa, reach the dentin by migrating into the gingival sulcus and pass painlessly to the target site. On reaching the dentin, the nanorobots enter dentinal tubule holes that are 1 to 4 Am in diameter and proceed toward the pulp, guided by a combination of chemical gradients, temperature differentials, and even positional navigation, all under the control of the onboard nanocomputer as directed by the dentist.

Nanotechnology in orthopedic applications:

Biomaterials proposed as ideal scaffolds for cell growth should be biocompatible, osteoinductive, osteoconductive, integrative, porous, and mechanically compatible with native bone to fulfill their desired role as bone implants and substitutes.

Nanostructure materials with sizes 1 to 100 nm can act as new and effective constituents of bone materials, because bone is also made up of nanosized organic and mineral phases.

Nanomaterials, nanopolymers, carbon nanofibers, nanotubes, and nanocomposites of ceramics will also lead to more efficient deposition of calcium containing minerals on the implants.

Nanotechnology as a risk to human health:

Nanomaterials can enter the human body through several ports. Accidental or involuntary contact during production or use is most likely to occur via the lungs, from which a rapid translocation is possible to other vital organs through the bloodstream. On the cellular level, an ability to act as a gene vector has been demonstrated for nanoparticles. Carbon black nanoparticles have been implicated in interfering with cell signaling. There is work that demonstrates uses of DNA for the size separation of carbon nanotubes. The DNA strand just wraps around it if the tube diameter is right. Though excellent for the purposes of separation, this tendency raises some concerns over the consequences of carbon nanotubes entering the human body. (S K Sahoo et al., 2007).

CHAPTER II

A REVIEW – POLYMERIC NANOPARTICLES

CHAPTER II

REVIEW ON POLYMERIC NANOPARTICLES

Introduction:

The field of polymer nanoparticles is quickly expanding and playing a pivotal role in a wide spectrum of areas ranging from electronics to photonics, conducting materials to sensors, medicine to biotechnology, pollution control to environmental technology, and so forth, during the past decades.

Polymeric nanoparticulate systems from biodegradable and biocompatible polymers are interesting options for controlled drug delivery and drug targeting. Polymer nanoparticles are solid colloidal particles with diameter ranging from 1 to 1000nm. They have been investigated especially in drug delivery and drug targeting owing to their particle size and long circulation in the blood. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached.

Nanoparticles have been widely used in biomedical applications due to their specific physical and chemical properties which alter the normal biological activity, as compared to bulk materials. Science-based definition must be developed by several national and international standardization bodies, as well as organizations and authorities in order to have a definition that is broadly applicable to regulatory legislations.

Polymeric nanomaterials offer a promising solution by encapsulating chemotherapy drugs, and have been shown to reduce toxicity by providing a protective housing for the drug that limits its interaction with healthy cells. As a result, the pharmacokinetic properties of the drug are based on the pharmacokinetic

properties of the particle, as long as the drug can stay entrapped with the carrier until release is desired. The potential benefits of such delivery devices also include controlled and long-term release rates, prolonged bioactivity, reduced side effects, increased patient compliance due to decreased administration frequency, and the ability to codeliver multiple drugs with synergistic effects to the same site.

Current research has thus focused on advancing these polymer vehicles with "smart" technologies that are responsive to environmental stimuli. These can be separated into two categories: (1) site-targeting, where particles actively search for and attach themselves to specific and diseased cells by the use of molecules such as ligands, antibodies, and aptamers; (2) site-triggering, where chemical or physical changes in the environment trigger the rapid release of the drug payload. This review focuses on a few selected "smart" technologies in each category: ligand and aptamer site-targeting particles, and pH and temperature-responsive particles. (Kurt E Geckeler et al., 2011 Erik brewer et al., 2011 Aura Ileana Moreno Vega et al.,2012).

Advantages of polymeric nanoparticles:

a) Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
b) They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
c) Delivers a higher concentration of pharmaceutical agent to a desired location.
d) The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.
e) Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering. (Hemant K.S.Yadav et al., 2012).

Types of Polymeric nanoparticles:

- **Nanospheres:**

  Nanospheres are matrix particles, i.e., particles whose entire mass is solid and molecules may be adsorbed at the sphere surface or encapsulated within the particle.

- **Nanocapsules:**

  Nanocapsules are vesicular systems, acting as a kind of reservoir, in which the entrapped substances are confined to a cavity consisting of a liquid core (either oil or water) surrounded by a solid material shell.

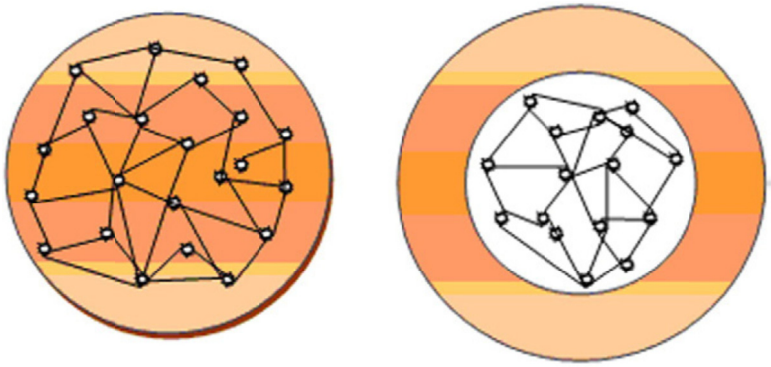


FIGURE 13: STRUCTURE OF NANOPARTICLES A) NANOSPHERE B) NANOCAPSULE

Polymers used in preparation of nanoparticles:

The polymers should be compatible with the body in the terms of adaptability (non-toxicity) and (non-antigenicity) and should be biodegradable and biocompatible.

Natural polymers:

The most commonly used natural polymers in preparation of polymeric nanoparticles are

- Chitosan
- Gelatin

- Sodium alginate
- Albumin

There are many **synthetic polymers** like

- Polylactides(PLA)
- Polyglycolides(PGA)
- Poly(lactide co-glycolides) (PLGA)
- Polyanhydrides
- Polyorthoesters
- Polycyanoacrylates
- Polycaprolactone
- Poly glutamic acid
- Poly malic acid
- Poly(N-vinyl pyrrolidone)
- Poly(methyl methacrylate)
- Poly(vinyl alcohol
- Poly(acrylic acid)
- Poly acrylamide
- Poly(ethylene glycol)
- Poly(methacrylic acid)

(Hemant K.S.Yadav et al., 2012)

- **Preparation techniques for polymer nanoparticles:**

Polymeric nanoparticles can be conveniently prepared either from preformed polymers or by direct polymerization of monomers using classical polymerization or polyreactions.

Methods like solvent evaporation, salting-out, dialysis and supercritical fluid technology, involving the rapid expansion of a supercritical solution or rapid expansion of a supercritical solution into liquid solvent, can be utilized for the preparation of polymeric nanoparticle from preformed polymers.

On the other hand, polymeric nanoparticles can be directly synthesized by the polymerization of monomers using various polymerization techniques such as micro emulsion, mini-emulsion, surfactant-free emulsion and interfacial polymerization.

The choice of preparation method is made on the basis of a number of factors such as the type of polymeric system, area of application, size requirement, etc. For instance, a polymeric system that is developed for an application in the biomedical or environmental fields should be completely free from additives or reactants such as surfactants or traces of organic solvents. In this case, techniques like RESS (rapid expansion of a supercritical solution) or RESOLV (rapid expansion of a supercritical solution into a liquid solvent) can be selected, as they do not utilize any surfactant or organic solvent during the polymeric nanoparticles preparation.

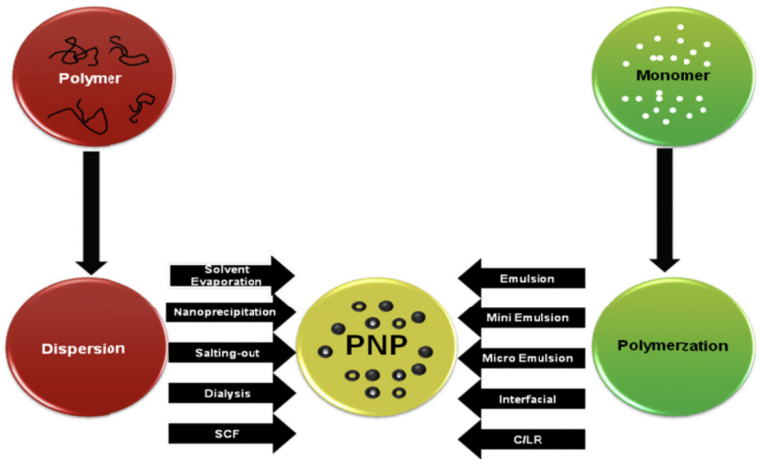


FIGURE 13: SCHEMATIC REPRESENTATION OF VARIOUS TECHNIQUES FOR THE PREPARATION OF POLYMERIC NANOPARTICLES. SCF: SUPERCRITICAL FLUID TECHNOLOGY, C/LR: CONTROLLED/LIVING RADICAL

Dispersion of preformed polymers:

- **Solvent evaporation:**

Solvent evaporation was the first method developed to prepare PNPs from a preformed polymer. Although originally proposed by polymer chemists, its main developments are found in pharmaceutical technology where biodegradable polymers have been applied in the production of drugs. In this method, polymer solutions are prepared in volatile solvents and emulsions are formulated. In the past, dichloromethane and chloroform were widely used, but are now replaced with ethyl acetate which has a better toxicological profile. The emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion.

Solvent evaporation is the most widely employed technique to prepare nanoparticles of polymers in the current literature on techniques using a dispersion of preformed polymers. (Kurt E Geckeler et al., 2011).

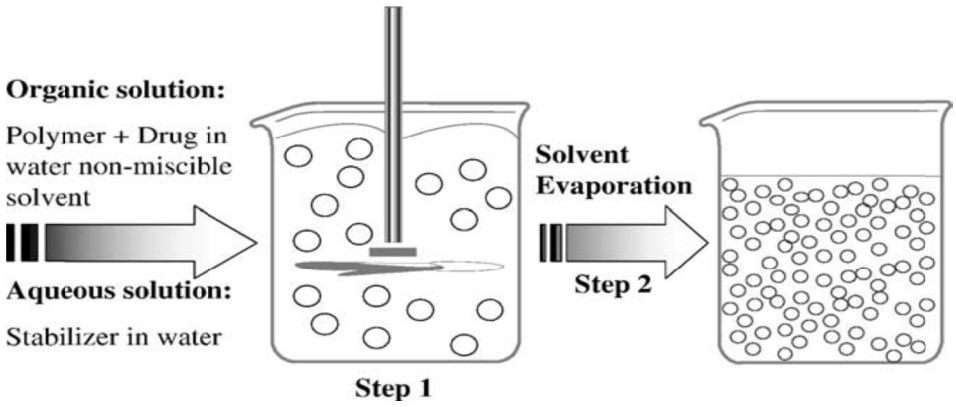


FIGURE 14: SCHEMATIC REPRESENTATION OF THE SOLVENT EVAPORATION METHOD

- **Salting-out:**

Salting -out is based on the separation of a water miscible solvent from aqueous solution via a salting-out effect. The salting-out procedure can be considered

as a modification of the emulsification/solvent diffusion. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non- electrolytes such as sucrose) and a colloidal stabilizer such as polyvinylpyrrolidone or hydroxyethylcellulose. This oil/water emulsion is diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres. The selection of the salting out agent is important, because it can play an important role in the encapsulation efficiency of the drug. Both the solvent and the salting-out agent are then eliminated by cross-flow filtration.

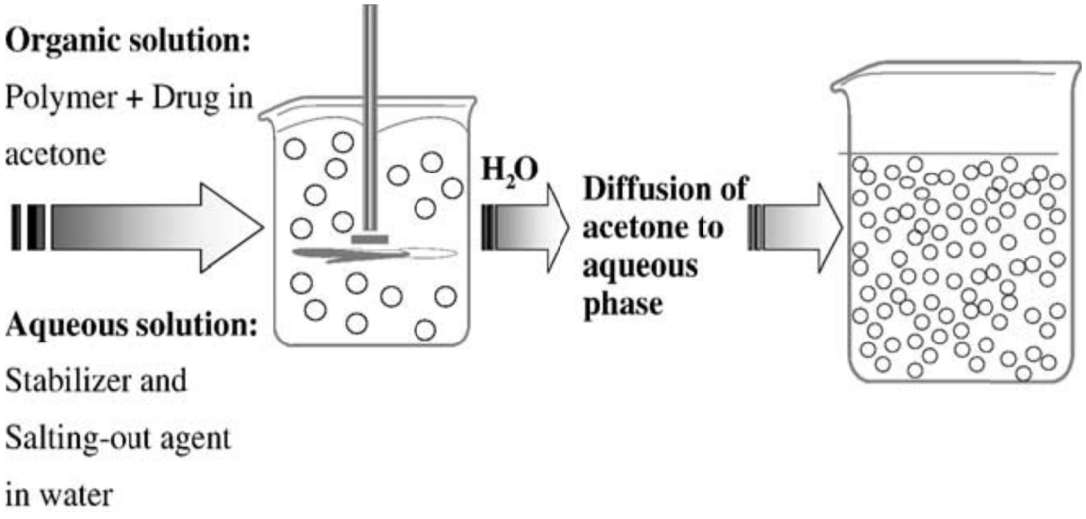


FIGURE 15: SCHEMATIC OF THE SALTING-OUT TECHNIQUE.

- **Nanoprecipitation:**

The nanoprecipitation method was developed by Fessi et al for the preparation of polymeric nanoparticles. It is also called as solvent displacement method. The basic principle of this technique is based on the interfacial deposition of a polymer after displacement of a semipolar solvent, miscible with water, from a lipophilic solution. Rapid diffusion of the solvent into non-solvent phase results in the decrease of

interfacial tension between the two phases, which increases the surface area and leads to the formation of small droplets of organic solvent.

Nanoprecipitation system consists of three basic components: the polymer (synthetic, semi synthetic or natural), the polymer solvent and the non-solvent of the polymer. Organic solvent (i.e., ethanol, acetone, hexane, methylene chloride or dioxane) which is miscible in water and easy to remove by evaporation is choosen as polymer solvent.

Due to this reason, acetone is the most frequently employed polymer solvent in this method. Sometimes, it consists of binary solvent blends, acetone with small amount of water, blends of acetone with ethanol and methanol. On the other hand, the non-solvent phase consisting of a non-solvent or a mixture of non-solvents is supplemented with one or more naturally occurring or synthetic surfactants.

The key variables determining the success of the method and affecting the physicochemical properties of polymeric nanoparicles are those associated with the conditions of adding the organic phase to the aqueous phase, such as organic phase injection rate, aqueous phase agitation rate, the method of organic phase addition and the organic phase to aqueous phase ratio.

Likewise, polymeric nanoparticles characteristics are influenced by the nature and concentration of their components. Although, a surfactant is not required to ensure the formation of polymeric nanoparticles by nanoprecipitation, the particle size is influenced by the surfactant nature and concentration.

Moreover, the addition of surfactants helps to preserve the nanoparticle suspensions from agglomeration over long storage periods. (Kurt E Geckeler et al., 2011).

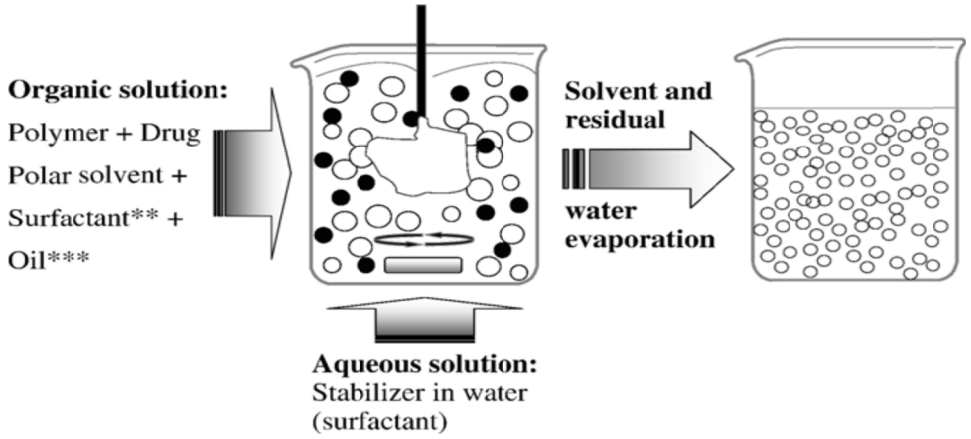


FIGURE 16: SCHEMATIC REPRESENTATION OF THE SOLVENT DISPLACEMENT/INTERFACIAL DEPOSITION TECHNIQUE

- **Dialysis:**

Dialysis offers a simple and effective method for the preparation of small, narrow-distributed polymeric nanoparticles. Polymer is dissolved in an organic solvent and placed inside a dialysis tube with proper molecular weight cutoff. Dialysis is performed against a non-solvent miscible with the former miscible. The displacement of the solvent inside the membrane is followed by the progressive aggregation of polymer due to a loss of solubility and the formation of homogeneous suspensions of nanoparticles.

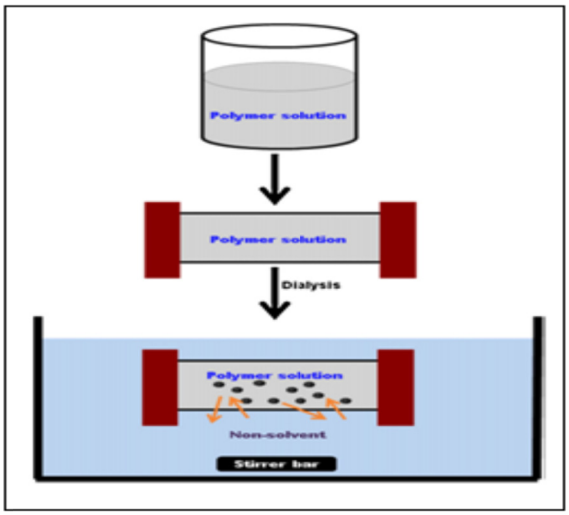


FIGURE 17: SCHEMATIC REPRESENTATION OF OSMOSIS BASED METHOD FOR PREPARATION OF POLYMER NANOPARTICLES.

- **Supercritical fluid technology:**

As may be noted, the methods in the preceding subsections involve organic solvents, and the need to develop environmentally safer methods for the production of polymeric nanoparticles has motivated research on the utility of supercritical fluids as more environmental friendly solvents, with the potential to produce polymeric nanoparticles with high purity and without any trace of organic solvent. Supercritical fluid and dense gas technology are expected to offer an interesting and effective technique of particle production, avoiding most of the drawbacks of the traditional methods. Indeed, examples have been published on pharmaceutical particle formation, formulation, and control with a supercritical fluid and dense gas.

Two principal processes have been developed for the production of nanoparticles using supercritical fluids:

1. Rapid expansion of supercritical solution (RESS)
2. Rapid expansion of supercritical solution into liquid solvent (RESOLV)

1. Rapid expansion of supercritical solution (RESS) :

In traditional RESS, the solute is dissolved in a supercritical fluid to form a solution, followed by the rapid expansion of the solution across an orifice or a capillary nozzle into ambient air.

The high degree of supersaturation, accompanied by the rapid pressure reduction in the expansion, results in homogenous nucleation and, thereby, the formation of well-dispersed particles.

Results from mechanistic studies of different model solutes for the RESS process indicate that both nanometer- and micrometer-sized particles are present in the expansion jet.

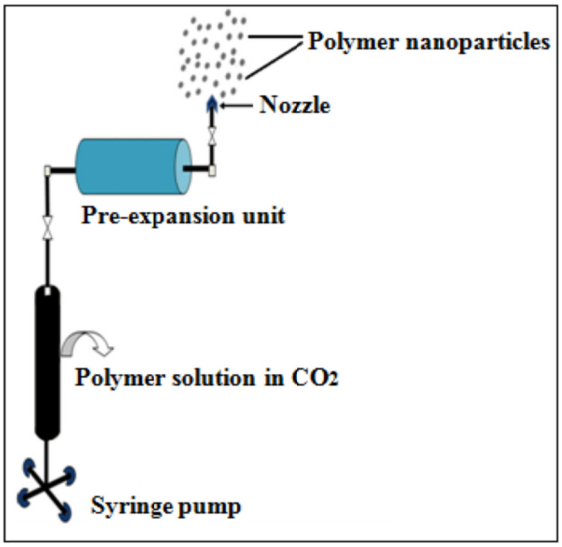


FIGURE 18: EXPERIMENTAL SET-UP FOR PREPARATION OF POLYMER NANOPARTICLES BY RAPID EXPANSION OF SUPERCRITICAL FLUID SOLUTION

2. Rapid expansion of supercritical solution into liquid solvent (RESOLV):

A simple, but significant modification to RESS involves expansion of the supercritical solution into a liquid solvent instead of ambient air, termed as RESOLV. The liquid solvent apparently suppresses the particle growth in the expansion jet, thus making it possible to obtain primarily nanosized particles. (Kurt E Geckeler et al., 2011).

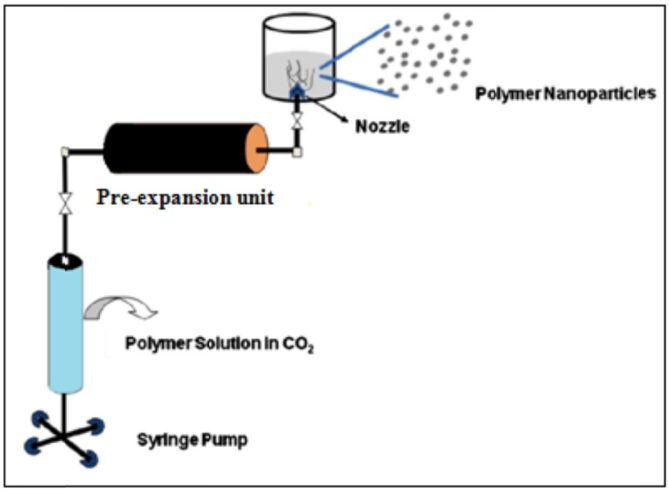


FIGURE 19: EXPERIMENTAL SET-UP FOR THE RAPID EXPANSION OF SUPERCRITICAL FLUID SOLUTION INTO LIQUID SOLVENT PROCESS

Physicochemical characteristics of polymeric nanoparticles:

- Particle size – Photon correlation spectroscopy,
  Transmission electron microscopy,
  Scanning electron microscopy,
  Scanned probed microscope,
  Fraunhofer diffraction,
  Freeze fracture electron microscopy.
- Molecular weight - Gel chromatography
- Density – Helium compression pycnometry
- Crystallinity – X-ray diffraction, Differential scanning calorimetry
- Surface charge – Zeta potential measurement,
  Electrophoresis,
  Laser droplet anemometry,
  Amplitude weighed phase structure determination.
- Hydrophobicity - Hydrophobic interaction chromatography,
  Contact angle measurement,
  Rose Bengal binding.
- Surface properties - Static secondary-ion mass spectroscopy
- Surface element analysis - X-ray photon spectroscopy,
  Molecular magnetic resonance,
  Fourier transform infrared spectroscopy.

Drug loading :

- Incorporation method : Incorporating at the time of nanoparticles production.

- Incubation method : Adsorbing the drug after the formation of nanoparticles by incubating the carrier with the concentrated drug solution.

Drug release and release kinetics:

- Release from the surface of particles
- Diffusion through the swollen rubbery matrix
- Release due to erosion

Administration of polymeric nanoparticles:

- Intravenous administration
- Passive targeting
- Active targeting
- Subcutaneous and intramuscular injection
- Oral administration
- Ophthalmic application
- Nanoparticles and targeted drug delivery
  i.e Cancer therapy in drug targeting.

Applications of nanoparticles in drug delivery

S.No	Application	Material used	Purpose
1	Cancer therapy	Hydrophobic lactate – containing polymers, Poly(N-(2-hydroxypropyl) methacrylamide oligolactates), Poly (N-(2-hydroxyehtyl)methacrylamide)-oligolactates	Targeting, reducing toxicity, enhanced uptake of antitumour agents, improved in vitro and invivo stability.
2	Intracellular targeting	Poly (alkyl cyanoacrylate) polyesters nanoparticles with anti-parasitic or antiviral agents	Targeting reticuloendothelial intercellular infections
3	Prolonged systemic circulation	Polyesters with adsorbed poly ethylene glycols or pluronics	Prolonged systemic effect, avoid by the uptake of reticuloendothelial system
4	Vaccine adjuvant	Poly (methyl methacrylate) nanoparticles with vaccines (oral and IM immunization)	Enhanced immune response, alternate acceptable adjuvant
5	Peroral absorption	Poly (methyl methacrylate) nanoparticles with proteins and therapeutic agents	Enhanced bioavailability, protection from GIT enzymes
6	Ocular delivery	Poly (alkyl cyanoacrylate) nanoparticles with steroids,	Improved retention of drug/ reduced

		anti- inflammatory agents, anti bacterial agents for glaucoma	wash out
7	DNA delivery	DNA-gelatine nanoparticles, DNA-chitosan nanoparticles, PDNA-poly (D,L-lactide-co-glycolide) nanoparticles	Enhanced delivery and significantly higher expression levels
8	Oligonucleotides delivery	Alginate nanoparticles, poly (D,L-lactic acid) nanoparticles	Enhanced delivery of oligonucleotides
9	Other applications	Poly (alkyl cyanoacrylate) nanoparticles with peptides, poly (alkyl cyanoacrylate) nanoparticles, Nanoparticles with adsorbed enzymes	Crosses BBB, Improved absorption and permeation for transdermal application, Enzyme immunoassays

(Erik brewer et al., 2011, Silvia S Guterres et al., 2007, Natarajan Jawahar et al., 2012).

CHAPTER III

LITERATURE REVIEW

CHAPTER III

LITERATURE REVIEW

Hadi Valizadeh et al., 2013, prepared Vancomycin loaded PLGA nanoparticles by double emulsion solvent evaporation method. The nanoparticles were characterized for their micrometritic and crystallographic properties, particle size, zeta potential, drug loading and release. Intestinal permeability of vancomycin-PLGA nanoparticles was determined in different concentrations using SPIP technique in rats. Particle sizes were between 450nm and 466nm for different compositions of vancomycin-PLGA nanoparticles. Entrapment efficiency ranged between 38.38% and 78.6% with negative zeta potential. The FT-IR, XRPD and DSC results ruled out any chemical interaction between the drug and PLGA. Effective intestinal permeability values of vancomycin nanoparticles in concentrations of 200, 300 and 400µg/ml were significantly higher than that of solutions at the same concentrations. The PLGA nanoparticles could provide a delivery system for vancomycin with enhanced intestinal permeability.

Ping Yao et al., 2013, prepared Doxorubicin loaded Biocompatible and biodegradable nanoparticles by BSA–dextran–folic acid conjugate via a pH adjustment and heating process. The BSA–dextran–folic acid conjugate was produced by an esterification reaction between folic acid and dextran and then the maillard reaction between the modified dextran and BSA. The nanoparticles have a size about 90nm and excellent dispersibility at pH 7.4 aqueous solution. The doxorubicin loading efficiency and loading amount of the nanoparticles are larger than 90% and 14%, respectively. The antitumor activity and toxicity of the nanoparticles were evaluated through murine ascites hepatoma H22 tumor-bearing mice. The nanoparticles allow the administration of the doxorubicin with higher

dose. At doxorubicin dose of 10 mg/kg, the nanoparticles can achieve 88.9% of the tumor inhibition rate that is the same as the free doxorubicin at the dose of 5 mg/kg. Importantly, the nanoparticles can decrease the toxicity of doxorubicin that results in a significant increase of the average life time in comparison with the free doxorubicin as well as the nanoparticles without folic acid.

Aravind Gulbake et al., 2013, developed Mesalazine (MSZ) loaded Chitosan nanoparticles (CH-NPs) by ionotropic gelation method. Encapsulated in Eudragit S100 coated pellets for site specific delivery to ulcerative colitis (UC). The CH-NPs were characterized for size and structure using Malvern zetasizer and transmission electron microscope (TEM). The average size of the uncoated CH-NPs was about 157.3±7.1 nm, with the zeta potential of 32.2±2.1 mV, suitable for uptake through the colonic mucosa due to their nano size range and mucoadhesive properties. The *in vitro* drug release from developed formulations was investigated using a USP dissolution rate test paddle-type apparatus in different simulated gastrointestinal tract fluids. The coated formulation shows no release, and uncoated CH-NPs showed 4.98±0.24 % of mesalazine in SGF pH 1.2. This suggests that the release of drugs from coated nanoparticles was pH-responsive. At the end of 24hours 69.24±3.4% and 45.26±2.4% of mesalazine was released from CH-NPs and EC-CH-NPs. The mesalazine and pellets of CH-NPs and EC-CH-NPs bearing mesalazine were separately administered orally at the dose of 50 mg/kg body weight to albino rats and evaluated for antiulcerogenic activity.

Krishna Sailaja A et al., 2013, prepared Nimesulide loaded cellulose acetate hydrogen phthalate nanoparticles by salting out technique. Nanoparticles were evaluated for particle size, zetapotential and particle size distribution. Size of the particle was measured by Scanning electron microscope. Surface charge and

stability of the resultant nanoparticles was determined by Zetasizer. Particle size distribution was determined by Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer Nano-ZS. The cellulose acetate hydrogen phthalate concentration and nimesulide concentration was varied from 5mg/ml to 10 mg/ml. The effect of drug and polymer concentrations on nanoparticle size, shape, particle size distribution was studied. Increased drug concentration has no impact on the particle size. The size of the particle was found to be decreased with increased polymer concentration. Increased polymer concentration has resulted in uniform particle size distribution. Higher the polymer concentrations and lower the drug concentrations resulted in uniform particle size distribution.

Vyjayanthimala T et al., 2013, formulated Stavudine loaded chitosan and eudragit nanoparticles by emulsion droplet coalescence method. The nanoparticles were evaluated for morphology, loading efficiency and *in-vitro* release. The particle shape and morphology of the prepared stavudine nanoparticles were determined by SEM analysis. The amount of Stavudine entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticle and the amount of non-entrapped drug remaining in the aqueous supernatant. A Franz diffusion cell was used to monitor stavudine release from the nanoparticles. The formulations CF1, CF2, EF2 and EF3 showed good drug release from the polymer . The percentage cumulative drug release after 12 hours was 75.54, 75.89, 78.86 and 76.42% respectively. However about 15% initial burst release was found at 1 hour in all formulations. EF2 released 78.86% of stavudine 12 hours with a burst drug release nearly 14.86% of drug within the initial 1 hour. Formulations 4 out of 8 showed good drug release from the polymer, the percentage cumulative drug release after 12 hours were in the range

of 72-78 %. Among the four formulations EF 2 (1% Chitosan & 1.5 % EudragitS 100) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency. *In-vitro* antiviral study revealed thatthe formulated nanoparticles were found to have good viral activity on viral cells in sustained manner.

Umar Faruksha A et al., 2013, formulated Pioglitazone Hydrochloride loaded Eudragit RL 100 nanoparticles by emulsification solvent evaporation method. The application of factorial design gave a statistically systematic approach for the formulation of nanoparticles. Nanoparticles were characterized by Differential scanning calorimetry (DSC) and Scanning electron microscopy (SEM). Drug content, entrapment efficiency and particle properties such as size, size distribution and zeta potential were determined. The designed nanoparticles have particle size from 136.7nm to 264.1nm and entrapment efficiency from 60.98% to 76.41%. Nanoparticles revealed a fast release during the first hour followed by a more gradual drug release during a 24 hours period following a Fickian diffusion process. 3^2 factorial design thus facilitated the optimization of polymeric nanoparticulate carrier systems for sustained oral delivery of the drug.

Srinivas P et al., 2012, formulated Moxifloxacin Hydrochloride loaded Eudragit RL 100 ocular nanoparticles by solvent displacement method. Different formulations by varying the ratios of drug and polymer and varying the ratios of organic and aqueous phase. The formulations were evaluated in terms of particle size, Fourier transform infrared spectroscopy (FTIR), drug entrapment efficiency. The mean particle size for drug loaded formulations was found to be below 200nm. The zeta potential remained in the range of positive values for all batches +10mv to 40mv. The invitro release studies suggest that release rate was related

the drug:polymer ratio. Increase of drug release was observed as a function of drug:polymer ratio. In vivo studies were performed on New Zeland albino rabbits. No ocular damage or abnormal clical signs to the cornea, iris or conjunctiva was visible. The most suitable storage condition for nanoparticles of moxifloxacin hydrochloride was at 4^0C.

Amulyaratna Behera et al., 2012, formulated Glibenclamide loaded poly (lactic-co-glyclolic) acid (PLGA) nanoparticle by solvent evaporation technique using methanol/dichloromethane (2:1) and characterized by Differential scanning calorimetry (DSC) and Transmission electron microscopy (TEM). The effect of stirring speed (250, 1000, 1500 and 2500 rpm) and drug:polymer ratio (1:1, 1:2, 1:3 and 2:1) on particle size, size distribution, zeta potential drug loading, encapsulation efficiency and drug release was also studied. Stable Nanoparticles were successfully prepared without any incompatibility, as indicated by DSC and TEM studies, respectively. As polymer and drug concentrations, and stirring speed increased, particle size, drug loading and encapsulation efficiency also increased. Increase in polymer concentration sustained drug release but reverse was obtained as drug concentration increased. Controlled release biodegradable Glibenclamide nanoparticles can be efficiently prepared by emulsification solvent evaporation method suitable modulating processing variables.

Kazutaka Higaki et al., 2012, formulated Paclitaxel loaded Polyethylene glycol and Polylactic acid block copolymer (PN-PTX) nanoparticles by emulsion solvent diffusion method. The physicochemical properties of PN-PTX prepared were assessed the mean particle size was around 80nm and the zeta potential was found to be almost netural. The invitro Paclitaxel release preoperty were assessed by a dialysis method. Paclitaxel was stably incorporated in polymeric

nanoparticles for a long time in the presence of serum. The in vivo pharmacokinetics of PN-PTX after intravenous administration was investigated in colon 26 tumor bearing mice. The invivo disposition characteristics of PN-PTX were very favourable, then evaluated the anti tumor effect of PN-PTX in C_{26} tumor bearing mice. It is considered that the favourable pharmacokinetic properties of nanoparticles and the drug incorporated do not always lead to its potent in vivo pharmacological activity, suggesting the importance of Paclitaxel release properties with in tumor tissues.

Dianrui Zhang et al., 2012, prepared Riccardin D nano suspension by evaportative precipitation into aqueous solution and the microfluidisation process. The characteristics of transmission electron spectroscopy, size distribution and zeta potential. In the evaporative precipitation in to aqueous solution method, the drug was dissolved in the organic phase and F68, HPMC, PVP K 30 were dissolved in water the mass ratio of 2:1:2:1. Differential Scanning Calorimetry (DSC) and X-Ray diffraction confirmed the crystalline states that were both reserved. The solubility was greatly improved by the two methods and the EPAS nanocrystals were more soluble due to the smaller size. An enhanced dissolution was obvious in vitro and the stable nanocrystals were successfully achieved by the two methods.

Mahmood Alaei – Beirami et al., 2012, prepared Diclofenac Sodium loaded Eudragit RS 100 nanoparticles using nanoprecipitation – solvent deposition technique (the single emulsion technique). Particle size and size distribution of nanoparticles were studied by applying laser diffraction particle size analyzer, and morphology of the nanoparticles was also inspected by transmission electron microscopy (TEM). All the prepared formulation using eudragit RS 100 resulted

in nano-range size particles with relative spherical smooth morphology and drug loading efficiency of nearly 100%. Accroding to these findings, nanoprecipitation – solvent deposition technique was able to engineer diclofenac sodium – eudragit Rs 100 nanoparticles to reach target size that could undergo more studies for evaluation and comparison of the anti inflammatory effect of drug in nanoparticles with classical dosage forms following its ocular administration.

Bharathi M et al., 2012, prepared valsartan loaded eudragit L 100 nanoparticles by nanoprecipitation method. The nanoparticles were characterized by FTIR, DSC, SEM, Particle size analysis, Invitro diffusion and invivo studies are been performed. The particle sizes of the nanoparticles were ranging from 175nm to 232nm. The formulation F2 in best drug release 60.38% at the end of 24 hours. Invivo studies revealed that in case of free drug, 40.9mcg/ml drug of maximum dose was recovered but in case of nanoparticles the dose recovered in serum was 16.02mcg/ml after 6 hours. The formulation stored at $4^0 \pm 1^0$C was more stable compared to the other temperatures. The feasibility of formulating valsartan loaded eudragit L 100 nanoparticles for the treatment of hypertensionby enhancing the bioavailability.

Sankar V et al., 2012, formulated Zidovudine-Lamivudine loaded Poly (lactic-co-glycolic acid) PLGA (50:50), Poly (lactic acid) PLA, Poly (methyl methacrylate) PMMA, methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) by emulsion polymerization method. The particle size and the surface morphology results revealed that PLGA nanoparticles (NPs) were smooth spherical with a size ranging from 58-224nm. The drug content in lyophilized PLGA NPswas found to be 51.67% (Zidovudine) 58.33% (Lamivudine) and no drug loss was found after storage for 1 month at room temperature. Invitro release

studies revealed that the rate of drug release from PLGA NPs was 95.38% in 10 hours with zidovudine, and 97.37% in 10 hours with lamivudine which was slower, when compared to MMA-SPM, PLA and PMMA NPs. The rate of drug release from MMA_SPM NPs was 64.33% in 10 hours with zidovudine and 95.43% in 10 hours with lamivudine. Acute toxicity studies in mice revealed that the dose administered doses not induce mortality in test animal.

Nepolean R et al., 2012, formulated Nisoldipine loaded Eudragit S 100 nanoparticles by nanoprecipitation method. The interactions between the drug and polymer were investigated by Fourier transform infrared spectroscopy (FTIR) and Diffrential scanning colorimetry (DSC). In vitro characteristics like particle size and size distribution, surface morphology and structural characterization, surface charges, drug content, entrapment efficiency, loading capacity and pH dependent drug release. The nanoparticles were in uniform shape, narrow size distribution with an average size of about 400nm. In vitro release of Nisoldipine nanoparticles was found to be pH responsive and is evident for the controlled release of its payload only at colon to increase the bioavailability of Nisoldipine by evading the cytochrome P 450 induced metabolism in liver and gut wall. The experimental results indicate that Nisoldipine loaded Eudragit S 100 nanoparticles have better physicochemical characteristics and can be used as a drug carrier for targeted delivery of Nisoldipine in colon, in order to enhance its oral bioavailability.

Mukesh S Patil et al., 2011, prepared and Optimized Simvastatin nanoparticles by nanoprecipitation method using partially water miscible solvents and the mutual saturation of the aqueous and organic phases prior to form a nanosuspension in order to reduce the initial thermodynamic instability of the nanoparticles. It was possible to prepare aqueous dispersions of colloidal size

containing upto 30% w/v of Eudragit L 100 using methanol as a water miscible solvent with surfactant. Simvastain was formulated as a nanoparticles in an attempt to increase its solubility and bioavailability. An optimized formulation of nanoparticles containing Simvastatin was developed through the experimental design, particle size analysis and zeta potential on oral administration in rats, nanoparticles provided significant increase in the bioabilability compared to a powder suspension formulation. The nanoparticles have a higher surface to volume ratio as compared with bulk material and therefore the dose and frequency of administration would be reduced hence increasing patient compliance.

Poovi G et al., 2011, formulated and optimized Repaglinide loaded Chitosan nanoparticles by solvent evaporation method in three different ratios. The prepared nanoparticles were evaluated for particle size, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy study (FT-IR), percentage yield, drug entrapment and for in vitro release kinetics. Scanning Electron Microscopy exposed that nanoparticles were spherical in shape with a nearly smooth surface morphology. Particle size was analyzed by Malvern particle size analyzer and shown 48-100nm range. FT-IR Study reveals that, there was no interaction between Repaglinide and polymers. The invitro drug release data to various kinetic equations indicated first order release, swelling and diffusion mechanism from Repaglinide nanoparticles. The bioavailability of drug may be improved and may help to reduce the dose of the drug and frequency administration. Controlled drug delivery system of poorly water soluble drugs like Repaglinide.

Liang Fang et al., 2011, prepared Revaprazan Hydrochloride nanosuspension by high pressure homogenization technique. Their crystalline state were evaluated by

DSC and Powder X-ray diffraction were used to study crystalline state of freeze dried powder of Revaprazan Hydrochloride suspensions. The results showed that particles of Revaprazan Hydrochloride microsuspension and nanosuspension remained in the same crystalline state as coarse suspension. The invitro dissolution test, both microsuspension and nanosuspension showed increased dissolution rate. In vivo studies indicated that only nanosuspension could significantly increase oral bioavailability of Revaprazan Hydrochloride in rats. That means, in the case of particle size reduction, enhanced oral bioavailability can be achieved by reducing Revaprazan Hydrochloride particle size into nano range.

Amar Singh et al., 2011, formulated Losartan Potassium loaded Chitosan nanoparticles by ionic gelation of chitosan with tripolyphosphate anions. Nanoparticles of different core:coat ratio were formulated and evaluated for drug content, loading efficiency, particles size, zeta potential, invitro drug release and stability studies. Scanning Electron Microscopy indicated that the nanoparticles were found to be in nanometer range and showed ideal surface morphology. Differential Scanning Calorimetry analysis indicated that there were no chemical interactions between drug and polymer and stability of drug, in vitro release behaviour from all the drug loaded batches were found to follow zero order and provided sustained release over a period of 24 hours. The developed formulation overcomes and could possibility be advantageous in terms of sustained release dosage forms of losartan potassium.

Gaurav K Jain et al., 2011, prepared Atorvastatin loaded Chitosan nanoparticles by high pressure homogenization (HPH) method. Scanning Electron Microscopy (SEM) revealed that Chitosan – Atorvastatin nano conjugate process smooth

surface where as X-ray diffraction (XRD) spectra demonstrated amorphous nature of nano conjugate. Chitosan – Atorvastatin nano conjugate showed solubility enhancement of nearly 4 – fold and 100 – fold compared to Chitosan – Atorvastatin conjugate and pure Atorvastatin. In vitro drug release studies in simulated gastric fluid and simulated intestinal fluid suggested sustained release of Atorvastatin from the conjugate. The plasma concentration time profile of Atorvastatin after oral administration of CH – AT nano – conjugate (2574±95.4 ng/mL) to rat exhibited nearly 5 fold increase in bioavailability compared with AT suspension (583±55.5 ng/mL). AT suspension was also reduced when AT was administrated in form of CH – AT nano conjugate. Chitosan conjugate nano prodrugs may be used as sustained polymeric prodrugs for enhancing bioavailability.

Sowkar Baig et al., 2011, prepared Abacavir Sulfate loaded Alginates nanoparticles by in situ nano emulsion – polymer cross linking method. The nanoparticles using different ratios of alginates and abacavir sulphate (ag-abs) in the ratios of (1:1, 1:2 and 1:3). The encapsulation efficiency was also studied to find out the percentage drug entrapped in the prepared nanoparticles. The result of ratio (1:3) showed a good encapsulation efficiency of 98.71%. Abacavir Sulfate nanoparticle was confirmed by FT – IR, DSC and quantitated by UV prepared nanoparticle appeared spherical with a dense drug core in transmission electron microscopy studies. Hydro dynamic diameter of nanoparticles was 63±0.235nm, with a Gaussian distribution and the zeta potential -0.6mev sustained diffusive drug release was observed in vitro, following zero order kinetics releasing the drug pay load over a period of 16 hr. Embedding abacavir sulphate in alginate provided sustained release. They also effered better

pharmacokinetic properties to the drug than that afforded by the free drug it self. The nanoparticle technique developed can be a good choice for the development of sustained anti retroviral drug carrier.

Syam Potnuru et al., 2011, prepared Stavudine loaded Chitosan nanoparticles by solvent evaporation method, in situ nanoemulsion polymer cross linking method. In characterization the obtained particles size of nanoparticles is 65.5 – 176nm, size distribution and shape were done by using Scanning Electron Microscopy, zeta potential is for the best formulation. According to release characteristics following the zero order release kinetics and release 16 hours. Comparatively based upon the method of preparation in situ nanoemulsion polymer cross linking method and solvent evaporation method, the in situ method has produced a good results like drug loading efficiency, in vitro release studies, t_{90} , zeta potential and the corresponding formulation produced good results.

Ji Jingou et al., 2011, prepared Methotrexate and Calcium folinate loaded Chitosan and Cyclodextrin nanoparticles by cross linking method. The prepared nanoparticles were characterized by FT – IR Spectroscopy to confirm the cross linking reaction between chitosan and cross linking agent. X-ray diffraction (XRD) was performed to reveal the form of the drug after encapsulation. The average size of nanoparticles ranged from 308.4±15.22 to 369.3±30.01nm. The nanoparticles formed were spherical in shape with high zeta potentials (higher than +30mv). In vitro release studies in phosphate buffer saline (pH 7.4) showed an initial burst effect and followed by a slow drug release, cumulative release data were fitted to an empirical equation to compute diffusional exponent (n), which indicated the non Fickian trend for drug release.

Peng Liu et al., 2011, prepared Indomethacin and Itraconazole nanosuspension by wet milling technique. 4 types of stabilizers at 4 different concentrations were tested on 2 structurally different drug compounds. Photon Correlation Spectroscopy results showed that the finest nanosuspensions were obtained when 80 wt % (to drug content) Pluronic F 68 was the stabilizer for Indomethacin and 60 wt % Pluronic F 127 for Itroconazole compared physical mixtures, dissolution rates of the nanosuspensions showed significant increases. The morphology of nanoparticles was observed by Transmission Electron Microscopy. Crystalline state of the drugs before and after milling was confirmed using Differential Scanning Calorimetry and X-ray Powder Diffraction. The physical and chemical stabilities of the nanosuspensions after storage for 2 months at room temperature and 4^0C were investigated using TEM and HPLC. No obvious changes in particle size and morphology and no chemical degradation of the drug ingredients were seen.

Chi H Lee et al., 2011, prepared Sodium Fluorescein and Nile red loaded Eudragit S 100 nanoparticles by the modified quasi emulsion solvent diffusion method. The nanoparticles had homogeneous surface morphology with spherical nature and uniformed texture. In vitro release profile that model compounds were retained by the nanoparticles at vaginal pH. But they were rapidly released from particles at physiological pH. The nanoparticles cellular uptake by vaginal cells and subsequent drug release. No cytotoxicity of the nanoparticles was detected in vaginal cell lines. The pH sensitive Eudragit S 100 nanoparticles would be a potential carrier for not only topical delivery but also systemic delivery of therapeutically active compounds.

Suganeswari M et al., 2011, prepared Atorvastatin Calcium and Amlodipine Besylate nanoparticles by nanoprecipitation technique using PLGA, Eudragit RL Po as polymers and Pluronic F 68 as tribloere polymeric stabilizer. The preformulation studies were carried out to confirm the solubility studies, Hygroscopicity and loss on drying for drug identification. The prepared nanoparticles were assayed by HPLC to determine the drug content. The morphological shape was confirmed by using Scanning Electron Microscopy. The particle size distribution was analyzed by using particle size analyzer. The average mean particle size 550nm, 70nm, 80nm and 100nm respectively. In vitro release maximum drug entrapment efficiency for both drugs Atorvastatin and Amlodipine. In vivo release of drugs in a animal model. The nanoparticulate suspension of amlodipine is to improve its absorption rate and therapeutic efficacy.

Xuenong Zhang et al., 2011, prepared Cyclosporine A loaded Eudragit S 100 nanoparticles. The pharmacokinetic profile of freeze dried cyclosporine A Eudragit S 100 nanoparticles was studies with a random two way crossover study in dogs. The drug blood concentration was determined by internal standard HPLC method after oral administration of Cyclosporine A , Eudragit S 100 nanoparticles and Neoral. Pharmacokinetics parameters were calculated by 3P97 program. The concentration time data were fitted as a two compartment open model. The AUC of Cy-A-S-100 was higher than that of Neoral ($P<0.05$), while the CL significantly decreased ($P<0.05$). The relative bioavailability of Cy-A-S-100 nanoparticles were 135.9% compared with Neoral. The bioavailability of CyA was significantly improved Cyclosporine A loaded Eudragit S 100 nanoparticles

was a potential drug for developing a new Cyclosporine A nanoparticles solid formulation.

Khosro Adibkia et al., 2010, prepared Naproxen loaded Eudragit RS 100 nanoparticles by solvent evaporation/extraction technique (the single emulsion technique). The physicochemical characteristics of nanoparticles were studied applying particle size analysis, differential scanning calorimetry, X-ray crystallography, Fourier transform infrared spectroscopy and scanning electron microscopy. To improve the physicochemical characteristics of the drug. Agitation speeds of homogenizer and drug/polymer ratio have no significant effect on the size of the nanoparticles. The intermolecular interaction between Naproxen and Eudragit RS 100 was detected in the FT – IR Spectrum of the nanoparticles. It should be also evoked that all nanoparticles displayed a slowed release pattern with the reduced burst release in comparison with the intact drug powder and physical mixtures of drug and polymer.

Swarnali Das et al., 2010, prepared Amphotericin –B loaded Eudragit RL 100 nanoparticles by Solvent Displacement or Nanoprecipitation method. The formulations are evaluated in terms of particle size, zeta potential, and differential scanning calorimetry measurements. All the formulations remained with in a size range of 130nm to 300 nm in fresh preparation as well as after 2 months. The zeta potential was positive (+22 to +42mV) for all the formulations and was suitable for ophthalmic application. The nanosuspensions produced a sediment, which was easy to redisperse by simple hand agitation. No changes in macroscopic properties were observed. The nanoparticles showed good stability in 2^0 to 6^0 C and at room temperature. So they can be expected to be stable, safe and effective after long term storage. In vivo studies suggest that Amphotericin B nanoparticles

can be expected to gain considerable attention for ocular antifungal effect and aminimal eye irritating effect.

Bivash Mandal et al., 2010, prepared Sulfacetamide loaded Eudragit RL 100 nanosuspension by solvent displacement method using acetone and pluronic F 108 solution. Drug to polymer ratio was selected as formulation variable. Characterization of the nanosuspension was performed by measuring particle size, zeta potential, Fourier transform infrared spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), Powder X-ray Diffraction (PXRD), drug entrapment efficiency and invitro release. In addition, freeze drying, redispersibility and short term stability study at room temperature and at 4^0C were performed. Spherical, uniform partices (size range below 500nm) with positive zeta potential were obtained. No significant chemical interaction between drug. The prepared nanosupension exhibited good stability after storage at room temperature and at 4^0C. Sucrose and Mannitol were used as Cryoprotectants and exhibited good water dispersibility of the FDN. It could be utilized as potential delivery system for treating ocular bacterial infections.

Mishra B et al., 2010, formulated Lamotrigine nanosuspension by emulsification solvent diffusion method. Characterized of the nanosuspension Fourier transform infrared (FT-IR), Differential scanning calorimetry, Partical size, polydispersity index (PDI) and zeta potential. Nanoparticles were spherical with little surface adsorbed drug. Properties of nanosuspensions in terms of size, zeta potential, poly dispersity index, entrapment efficiency, drug content and in vitro drug release were consistent and with in their acceptable ranges. In vitro drug release studies suggested that nanosuspension might be used as a sustained delivery vehicle for Lamotrigine. Statistical analysis revealed that size of the nanoparticles was most

strongly affected by stabilizer type while entrapment efficiency was influenced by the drug to polymer ratio. Release rate seemed to be governed by rate of diffusion of drug from polymeric matrix. The formulation remained reasonably stable up to 3 months under stressed storage conditions.

Rezaei Mokarram A et al., 2010, prepared Indomethacin loaded Polyvinyl pyrrolidine (PVP) Nano Solid Suspension by controlled precipitation technique, characterized by differential scanning calorimetry (DSC), X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and evaluated for in vitro solubility and dissolution rate . Absence of thermal and diffractional peaks in DSC and XRD studies indicated that indomethacin interacts with PVP in solid phase. The solubility of indomethacin in nano solid suspension compared to crystalline form was increased to about four fold. It was found that particle size distribution depend to the polymer molecular weight and drug:polymer ratios. TEM results as amorphous nanosize particles in freeze dried powder. Enhanced solubility and dissolution rate of indomethacin compared to physical mixtures and crystalline form of indomethacin demonstrated that it interacts with PVA via hydrogen bond and probably forming eutectic mixture.

Mitra Jelvehgari et al., 2010, prepared Theophylline loaded poly (□-caprolactone) nanoparticles by using water in oil in water ($w_1/o/w_2$ double emulsion solvent diffusion / evaporation method) taking different ratios of drug/polymer. Solvent systems consist of ethyl acetate and dichloromethane for microspheres and nanospheres respectively. In the current study formulations were characterized by loading efficiency, yield, particle size, zeta potential, X-ray diffraction and differential scanning calorimetry (DSC). The XRD and DSC showed stable character of theophylline in the drug loaded microspheres. The

drug release was found to be diffusion and erosion controlled. The burst was significantly lower with composite microparticles and may be explained by lower diffusion of the drug from double polymeric wall formed by the nanoparticles matri followed by another diffusion step through the microparticle polymeric wall.

Anilkumar J Shinde et al., 2010, prepared Simvastatin loaded polylactic-co-glycolic acid nanoparticles by precipitation solvent deposition method using 3^2 full factorial design. The prepared formulations were further evaluated for drug content, invitro drug release pattern, short term stability and drug excipient interactions. The application of factorial design gave a statistically systematic approach for the formulation and optimization of nanoparticles with desired particle size and high entrapment efficiency. Drug:polymer ratio and concentration of stabilizer were found to influence the particle size and entrapment efficiency of simvastatin loaded PLGA nanoparticles. Invitro drug release study of selected factorial formulations. The release was found to follow first order release kinetics with Fickian diffusion mechanism for all batches. These results indicate that simvastatin loaded PLGA nanoparticles could be effective in sustained drug release for a prolonged period.

Rathi J C et al., 2009, formulated Lamivudine loaded polymethacrylic acid nanoparticles in different drug to polymer ratio by nanoprecipitation method. SEM indicated that nanoparticles have a discrete spherical structure without aggregation. The average particle size was found to be 121±8 - 403±4nm. The particle size of the nanoparticles was gradually increased with increase in the proportion of polymethacrylic acid polymer. FT-IR studies indicated that there was no chemical interaction between drug and polymer and stability of drug. The invitro release behaviour from all the drug loaded batches was found to be zero

order and provided sustained release over a period of 24 hours. The slow and constant release of Lamivudine from nanoparticles maintain constant drug plasma concentration thereby increasing therapeutic efficacy.

Yadav A V et al., 2009, formulated Carvedilol loaded Eudragit E 100 nanoparticles by the nanoprecipitation method using polymeric stabilizer poloxamer 407. Nanoparticles of carvedilol were obtained with high encapsulation efficiency, The particles were characterized for particle size by photon correlation spectroscopy and transmission electron microscopy. The particle size of the prepared nanoparticles ranged from 190nm – 270nm. Nanoparticles of carvedilol were obtained with high encapsulation efficiency 85-91%. This formulation approach can be used to improve the therapeutic efficacy of poorly soluble drugs.

Julijana Kristl et al., 2009, studied the advantages of Celecoxib nanosuspension by emulsion – diffusion method using three different stabilizers tween80, PVP K 30 and SDS. The nanosuspension characterized by particle size analysis, dissolution testing, scanning electron microscopy imaging, differential scanning calorimetry and X-ray powder diffraction. Spray dried nanosuspension was blended with microcrystalline cellulose, and compressed to tablets and their tensile strength, porosity and elastic recovery of tablets. The crystalline nano sized celecoxib alone or in tablets showed a dramatic increase of dissolution rate and extent compared to micronized. Markedly lower compaction forces are needed to compress tablets with nanosized compared to microsized celecoxib powder to produce tablets of equal tensile strength. Thus possibly improve their oral bioavailability.

Adlin Jino Nesalin J et al., 2009, formulated Flutamide loaded Chitosan nanoparticles by ionic gelation technique. Characterization of nanoparticles particle size analysis, estimation of percentage yield and drug loading capacity of chitosan nanoparticles, invitro drug release studies. SEM analysis their size distribution was found to be 400nm. The drug loading capacity of nanoparticles containing drug:polymer in various ratios. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. In vitro release of Flutamide showed a very rapid initial burst, and then followed by a very slow drug release. From the drug release studies it was observed that nanoparticles prepared with chitosan in the core:coat ratio 1:4 gives better sustained release for about 12 hours as compared to other formulations.

Sanjay Singh et al., 2009, prepared Risperidone loaded poly (D,L- lactide - co – glycolide) (PLGA) nanoparticles by nanoprecipitation method using polymeric stabilizer (Poloxamer 407). The nanoparticles were characterized for particle size by photon correlation spectroscopy and atomic force microscopy. The invivo efficacy (antipsychotic effect) of prepared formulations (nanoparticles and in situ gel containing nanoparticles) was studied by administering them subcutaneously to mice. Extrapyramidal side effects of the formulations were also studied. The particle size of the prepared nanoparticles ranged between 85 and 219 nm. About 89% to 95% drug encapsulation efficiency was achieved when risperidone was loaded at 1.7% to 8.3% by weight of the polymer. During in vivo studies prepared risperidone formulations showed n antipsychotic effect that was significantly prolonged over that of risperidone solution for up to 72 hours with fewer extrapyramidal side effects. The prolonged effect of risperidone was obtained

from the risperidone formulations administered subcutaneously, and this may improve the treatment of psychotic disorders by dose reduction.

Le Thi Mai Hoa et al., 2009, prepared Ketoprofen loaded Eudragit E 100 nanoparticles by emulsion solvent evaporation method. The morphology structure was investigated by scanning electron microscopy (SEM). The interactions between the drug and polymer were investigated by Fourier Transform Infrared Spectroscopy (FTIR). The size distribution was measured by means of Dynamic Light Scattering. The SEM observations give the surface morphological features, morphology of particles was spherical and homogeneous. The size distribution of the nanoparticles prepared was found in the range from 50 to 200nm, the mean diameter was 150nm. The interaction between the drug and the polymer was determined by Fourier Transform Infrared Spectroscopy. The carboxylic group of the Ketoprofen molecule interacts with the Eudragit.

Jawahar N et al., 2009, prepared carvedilol loaded PLGA nanoparticles by Nanoprecipitation method. Nanoparticles were examined for physicochemical characteristics, invitro release kinetics and invivo biodistribution studies. Average size of the nanoparticles were in range of 132-234nm. The drug encapsulation efficiency was 77.6% at 33% drug loading. Invitro cumulative release from the nanoparticles was 72% at 24 hours. Invivo biodistribution studies in rats revealed that these particles are distributed in heart, liver and kidney at higher concentration may allow their delivery to target sites. Sustained release of nanoparticles might extend the circulation time of drug will suitable for reducing the initial hypotensive peak and prolong the antihypertensive effect.

Weigen Lu et al., 2008, prepared Nimodipine nanosuspension compared with commercially available ethanol solution. Nimodipine nanosuspension by high pressure homogenization. The effects of the production parameters such as pressure, cycle numbers and crushing principles on the mean particle size, 99% diameter and polydispersity of the nanosuspension were investigated. Characterization of the product was performed by Scanning Electron Microscope (SEM) and Differential Scanning Calorimeter (DSC). The number of large particles in the Nimodipine nanosuspension was much fewer than that of the fat emulsions for parenteral nutrion. The saturation solubility was increased as the reduction of particle size into nanometer range, which led to the fast dissolution of drug nanocrystals. The aqueous nanosuspension might be a good choice for intravenously administrating poor soluble nimodipine, which is proved to have the lower intravenous irritation and incidence of phlebitis than ethanol product.

Rainer H Muller et al., 2008, prepared Ascorbyl Palmitate nanosuspensions by high pressure homogenization method. The physicochemical properties of Ascorbyl Palmitate nanosuspensions (mean nanocrystal size, zeta potential and chemical stability) were found to be dependent on the type of stabilizer (surfactant). It was found that the mean size of Ascorbyl Palmitate nanosuspensions stabilized with tween80 remained in the nanometer range and the amount of active determined by HPLC was more than 90% when stored at three different temperatures during 3 months. The obtained results after lyophilization revealed that the nanocrystal agglomeration of formulations lyophilized with out trehalose was more pronounced than those with trehalose. From the X-ray diffractorgrams, it was shown that Ascorbyl Palmitate remained in a crystalline

state which is physicochemically and thermodynamically more stable than Ascorbyl Palmitate in an amorphous state.

Ravikumar M N V et al., 2007, prepared Estradiol loaded PLGA Nanoparticles by emulsion diffusion evaporation method employing didodecyldimethyl ammonium bromide (DMAB) as stabilizer. The effect of polymer molecular weight and copolymer composition on particle properties and release behaviour. Drug release in vitro decreased with increase in molecular weight and lactide content of PLGA. In vivo data showed that with all the PLGA nanoparticulate formulations, same dose (1mg estradiol/rat) produced detectable blood levels for 5-11 days, depending on the molecular weight, copolymer composition and resultant particle size, compared to 1 day profile shown by pure drug. Thus, estradiol loaded PLGA nanoparticles can be effective in improving the oral bioavailability and decreasing the dosing frequency , there by minimizing the dose dependent adverse effects and maximizing the patient's compliance.

Annick Ludwig et al., 2006, prepared Ciprofloxacin loaded Eudragit Rs 100 or Eudragit RL 100 and PLGA nanoparticles by water - in - oil - in – water (w/o/w) emulsification and solvent evaporation, followed by high pressure homogenization. Two non biodegradable positively charged polymers, Eudragit RS 100 and RL 100, the biodegradable polymer poly (lactic - co - glycolic acid) or PLGA were used alone or in combination, with vaying ratios. The formulations were evaluated in terms of particle size and zeta potential. Differential Scanning Calorimetry measurements were carried out on the nanoparticles and on the pure polymers Eudragit and PLGA. Drug loading and release properties of the nanoparticles were examined. The antimicrobial activity aganist pseudomonas aeruginosa and staphylococcus aureus was determined. The mean diameter was

dependent on the presence of eudragit and on the viscosity of the organic phase. The zeta potential of all eudragit containing nanoparticles was positive in ultrapure water (around ±21/±25mV). The particles activity against P.aeruginosa and S.aureus was comparable with an equally concentrated ciprofloxacin solution.

Qiang Zhang et al., 2004, prepared Cyclosporine A loaded poly (methacrylic acid and methacrylate) copolymer nanoparticles using an adaptation of the quasi-emulsion solvent diffusion technique. The characterization and the dispersion state of CyA at the surface or inside the polymeric matrices of the nanoparticles were investigated. The invitro release studies were conducted by Ultracentrifuge method. The bioavailability of CyA from nanoparticles was neural microemulsion was assessed in Sprague- Dawley (SD) rats at a dose of 15mg/kg. The particle size of the nanoparticles was within the range from 37.4±5.6 to 106±14.8nm. The drug entrapped efficiency was very high and in all cases the drug was amorphous or molecularly dispersed within the nanoparticles polymeric matrices. In vitro release experiments revealed that the nanoparticles exhibited perfect pH dependant release profiles. The relative bioavailability of CyA was markedly increased by 32.5% for CyA - S 100 nanoparticles ($P<0.05$), and by 15.2% and 13.6% for CyA - L - 100 - 55 and CyA L 100 nanoparticles respectively. While it was decreased by 5.2% from CyA - E 100 nanoparticles when compared with the neural micromulsion with these results, the potential of pH sensitive nanoparticles for the oral delivery of CyA was confirmed.

Esko I Kauppinen et al., 2003, prepared Beclomethasone Dipropionate loaded Eudragit E 100 or Eudragit L 100 nanoparticles by a novel aerosol flo reactor method. Particle size and morphology, crystallinity, and thermal behaviour were determined as a function of particle composition. It was found that all the

nanoparticles produced, regardless of particle composition, had geometric number mean diameters of approximately 90nm, and were spherical showing smooth surfaces. The drug was molecularly dispersed in the amorphous polymeric matrix of the nanoparticles, and drug crystallization was not observed when the ambient temperature was below the glass transition temperature of the polymer.

Silvia S Guterres et al., 2003, prepared Diclofenac loaded miglylol 810 or benzyl benzoate and poly (ε-caprolactone) or Eudragit S 90 nanocapsule and nanosphere suspensions by nanoprecipitation and freeze dried after the addition of colloidal silicon dioxide. The powders were examined under scanning electron microscopy and gastro intestinal tolerance of products was evaluated in rats powders presented drug contents between 90.2±5.5% and 101.1±1.9% (HPLC). SEM analyzes showed non spherical microparticles and at higher magnifications, the micropowder surface presented a homogeneous nanocovering. Regarding the gastrointestinal tolerance, with the exception of benzyl benzoate loaded formulations powders presented loaded formulations powders presented lesional indexes lower than the diclofenac salt solution. In contrast to the literature, nano capsules can be dried by freeze drying with out leakage of drug or breaking the capsule wall.

Kristl J et al., 2002, prepared Enalaprilat loaded poly- (lactide - co - glycolide) (PLGA) and polymethyl methacrylate (PMMA) nanoparticles by the emulsification diffusion method. Characterized according to particle size, zeta potential, entrapment efficiency and physical stability. Effective permeabilities through rat jejunum of the enalaprilat in solution and in enalaprilat loaded nanoparticles were compared using side by side diffusion chambers. The solubility of enalaprilat is very low. The diameters of drug loaded PMMA and

PLGA nanoparticles were 297 and 204nm. The mean nanoparticle size and the polydispersity index were found to decrease with increasing PVA concentration. In vitro release studies show a biphasic release of enalaprilat from nanoparticle dispersions fast in the first step and very slow in the second. Ex vivo transport through isolated rat jejunum of enalaprilat in solution with that of enalaprilat in nanoparticle dispersions.

Snjezana Stolnik et al., 1999, prepared Procaine Hydrochloride loaded poly (DL - lactide - co- glycolide) (PLGA) nanoparticles by the nanoprecipitation method in water pH 5.8 as the aqueous phase. The nanoparticles produced were submicron size (<210nm) and low polydispersity. Drug content and drug entrapment were very low. This study therefore investigated the influence of various formulations variables on enhancing the incorporation efficiency of procaine hydrochloride. An increase in the aqueous phase pH from 5.8 to 9.3 enhanced the drug content and drug entrapment which may be due to a decreased degree of ionisation and hence lower solubility in the aqueous phase. Drug release from nanoparticles appears to consist of two components with an initial rapid release followed by a slower exponential stage. This study has demonstrated that formulation variables can be exploited in order to enhance the incorporation of a water soluble drug.

Philippe Maincent et al., 1998, prepared Isradipine loaded poly (epsilon - capro - lactone), poly (D,L - lactide) and poly (D,L - lactide - co - glycolide) by nanoprecipitation method. In vitro scanning electron microscopy and differential scanning calorimetry were used to characterize the nanoparticles. The average diameters of the nanoparticles ranged from 110nm to 208nm. The zeta potential of the nanoparticles was negative, with values of about -25mV which promoted good stabilization of the particles. X-ray diffraction and differential scanning

calorimetry have shown the amorphous state of PLA 100 and PLAGA nanoparticles and the semi crystalline state of PCL. In all types of nanoparticles non-crystalline isradipine was found in the polymer which suggests a molecular dispersion of the drug in the matrix. To reduce the initial hypotensive peak and to prolong the antihypertensive effect of the drug.

CHAPTER IV

AIM OF THE WORK

CHAPTER-IV

AIM AND OBJECTIVE OF THE WORK

Hypertension is one of the most common disorders throughout the world. Managing hypertension continues to be challenging with the currently available drugs, since they have poor bioavailability by oral route and due to toxicity at higher doses. The solubility and dissolution behaviour of a drug is the key determinant to its oral bioavailability. Improvement of oral bioavailability of poor water soluble drugs remains to be one of the most challenging aspects of drug development.

Felodipine is a BCS class II drug which has poor solubility and high permeability. It is a dihydropyridone calcium channel blocker which acts by decreasing smooth muscle contractility and subsequent vasoconstriction by inhibiting influx of calcium ions through voltage gated L-type calcium channels. Inhibition of initial influx of calcium decreases the contractile activity of smooth muscle cells and results in vasodilation, leading to overall decrease in blood pressure. Felodipine is used in the management hypertension. As given in conventional dosage form, oral bioavailability is very less i.e, 15% and the biological half life is 11 to 16 hours.

Nanoparticulate drug delivery system is one of the best approaches to enhance the dissolution rate and solubility of drugs suffer from oral bioavailability problems. Polymeric nanoparticles are the colloidal drug delivery system with a particle size of 10 – 1000 nm that potentially delivers the therapeutic agent in the systemic circulation in a controlled manner.

The main objective of this study is to formulate and evaluate oral nanoparticulate delivery of felodipine using the polymers eudragit L 100 and eudragit S 100 along with stabilizers pluronic F 68 and polyvinyl alcohol. And, this approach suggests that this polymeric nanoparticles will be a promising delivery system to improve the oral bioavailability as well as to maintain its therapeutic activity in a sustained release profile.

CHAPTER V

PLAN OF WORK

CHAPTER V

PLAN OF WORK

1. PREPARATION AND STANDARD CURVES FOR FELODIPINE:

a. Preparation of calibration medium

b. Estimation of absorption maximum (λmax)

c. Preparation of standard curve of Felodipine using Distilled water, 0.1N Hydrochloric acid, Phosphate buffer pH 6.5and Phosphate buffer pH 7.4

2. DRUG –POLYMER INTERACTION STUDIES:

a. Fourier Tranform Infrared spectroscopic analysis (FT-IR)

3. FORMULATION OF FELODIPINE LOADED POLYMERIC NANOPARTICLES

4. CHARACTERIZATION OF FELODIPINE LOADED POLYMERIC NANOPARTICLES:

a. Determination of drug content

b. Determination of drug entrapment efficiency

c. Determination of particle size, polydispersity Index & zeta potential

d. *In vitro* release studies of felodipine loaded polymeric nanoparticle using dialysis membrane

e. Kinetics of drug release studies

f. Selection and evaluation of best formulation

i. Lyophilization of nanosuspensions

ii. Infrared Spectroscopic analysis

iii. Solubility measurement studies

iv. Morphology of polymeric nanoparticles by SEM analysis

v. *Ex vivo* intestinal permeability studies

CHAPTER VI

MATERIALS AND EQUIPMENTS

CHAPTER VI

MATERIALS AND EQUIPMENTS

MATERIALS USED:

S.NO	MATERIALS	DISTRIBUTORS
1	Felodipine	Gift sample from Shasun Pvt Ltd, Pondicherry.
2	Eudragit L 100 (Polymethacrylic acid, methylacrylate,1:1)	Gift sample from Edict Pharmaceuticals, Chennai.
3	Eudragit S 100 (Polymethacrylic acid, methylacrylate,1:2)	Gift sample from Edict Pharmaceuticals, Chennai.
4	Pluronic F 68 (Poloxamer 188)	Gift sample from Madras Pharmaceuticals, Chennai.
5	PVA (Poly Vinyl Alcohol)	Gift sample from Shahsun Pharmaceuticals, Pondicherry.
6	Methanol	ChangshuYangyuah chemicals, China.
7	Monobasic Sodium Phosphate Monohydrate	Sigma Aldrich , USA.
8	Dibasic Sodium Phosphate anhydrous	High purity laboratory chemicals, Mumbai.
9	Sodium Lauryl Sulfate	High purity laboratory chemicals, Mumbai..
10	Sodium Hydroxide	High purity laboratory chemicals, Mumbai.
11	Hydrochloric Acid	Universal Scientifics, Madurai.
12	Sodium Chloride	High purity laboratory chemicals, Mumbai.

EQUIPMENTS USED:

S.NO	EQUIPMENTS	SUPPLIERS
1	Electronic weighing balance	A & D Company, Japan
2	UV-Visible spectrophotometer	Shimadzu Corporation, Japan
3	FT-IR (Fourier transform Infrared spectrophotometer)	Shimadzu, Japan.
4	Differential Scanning Calorimetry	DSC Q 200, Mumbai.
5	Homogenizer	M.S.E Ltd, England.
6	Refrigerator	Kelvinator, India.
7	Cooling Centrifuge Apparatus	Eppendorf Centrifuge 5417R, Germany
8	Scanning electron microscope	Hitachi X 650,Tokyo Japan.
9	Particle size analyser	Nano ZS 9o,Malvern Instruments Ltd U.K.
10	Environmental chamber	Inlab equipments Pvt. Ltd. Madras
11	Mechanical shaker	Secor, India.
12	Scanning electron microscope	Hitachi X650, Tokyo, Japan.
13	Ultra Sonicator	Vibronic's Ultrasonic processor, India.
14	Freeze dryer	Lyodel-Delvac Pumps Pvt. Ltd, USA.

CHAPTER VII

DRUG PROFILE

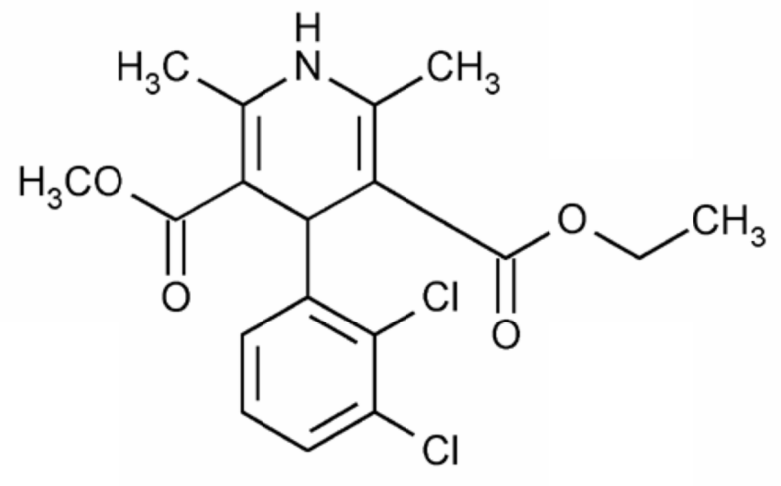
CHAPTER-VII

DRUG PROFILE

Synonym :

Felodipini; Felodipinum.

Structure :



Chemical formula : C_{18} H_{19} Cl_2 NO_4

Iupac name :

Ethyl methyl 4 – (2,3 – dichlorophenyl) – 1,4 dihydro – 2,6 – dimethyl pyridine – 3,5 - dicarboxylate.

Category :

Dihydropyridine calcium channel blocker, Vasodilator agents, Anti-hypertensive agents.

Indication:

For the treatment of mild to moderate essential hypertension**.**

Drug properties:

Nature :A white or light yellow, crystalline powder.

Melting point :145°.

Molecular weight :384.3.

Solubility : Freely soluble in methyl alcohol, dehydrated alcohol, in acetone and dichloromethane.

Log p :3.86

Half life :11 to 16 hours (oral administration in normal patients); 2 hours (patients with renal failure).

(Martindale 36th Edition ,Clarke's Analysis of Drugs and Poisons 3rd Edition & Drugbank.com).

Pharmacodynamics:

Felodipine belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), the most widely used class of CCBs. There are at least five different types of calcium channels in Homo sapiens: L-, N-, P/Q-, R- and T-type. It was widely accepted that CCBs target L-type calcium channels, the major channel in muscle cells that mediates contraction; however, some studies have shown that felodipine also binds to and inhibits T-type calcium channels. T-type calcium channels are most commonly found on neurons, cells with pacemaker activity and on osteocytes. The pharmacologic significance of T-type calcium channel blockade is unkown. Felodipine also binds to calmodulin and inhibits calmodulin-dependent calcium release from the sarcoplasmic reticulum. The effect of this interaction

appears to be minor. Another study demonstrated that felodipine attenuates the activity of calmodulin-dependent cyclic nucleotide phosphodiesterase (CaMPDE) by binding to the PDE-1B1 and PDE-1A2 enzyme subunits. CaMPDE is one of the key enzymes involved in cyclic nucleotides and calcium second messenger systems. Felodipine also acts as an antagonist to the mineralocorticoid receptor by competing with aldosterone for binding and blocking aldosterone-induced coactivator recruitment of the mineralocorticoid receptor. Felodipine is able to bind to skeletal and cardiac muscle isoforms of troponin C, one of the key regulatory proteins in muscle contraction. Though felodipine exhibits binding to many endogenous molecules, its vasodilatory effects are still thought to be brought about primarily through inhibition of voltage-gated L-type calcium channels. Similar to the DHP CCBs, felodipine binds directly to inactive calcium channels stabilizing their inactive conformation. Since arterial smooth muscle depolarizations are longer in duration than cardiac muscle depolarizations, inactive channels are more prevalent in smooth muscle cells. Alternative splicing of the alpha-1 subunit of the channel gives felodipine additional arterial selectivity. At therapeutic sub-toxic concentrations, felodipine has little effect on cardiac myocytes and conduction cells.(Drugbank.com).

Mechanism of action:

Felodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through voltage-gated L-type calcium channels. It reversibly competes against nitrendipine and other DHP CCBs for DHP binding sites in vascular smooth muscle and cultured rabbit atrial cells. Calcium ions entering the cell through these channels bind to calmodulin. Calcium-bound calmodulin then binds to and activates myosin light chain kinase (MLCK). Activated MLCK catalyzes the phosphorylation of the regulatory light chain subunit

of myosin, a key step in muscle contraction. Signal amplification is achieved by calcium-induced calcium release from the sarcoplasmic reticulum through ryanodine receptors. Inhibition of the intial influx of calcium decreases the contractile activity of arterial smooth muscle cells and results in vasodilation. The vasodilatory effects of felodipine result in an overall decrease in blood pressure. Felodipine may be used to treat mild to moderate essential hypertension.(Drugbank.com).

Pharmacokinetic properties:

Absorption :

Is completely absorbed from the gastrointestinal tract; however, extensive first pass metabolism through the portal circulation results in a low systemic availability of 15%. Bioavailability is unaffected by food.

Volume of Distribution :

Apparent volume of distribution, 10 L/Kg (normal patients); 5.6 L/Kg (patients with cirrhosis).

Protein binding:

Felodipine is approx 99% bound to plasma proteins, mainy albumin.

Metabolism :

Hepatic metabolism primarily via cytochrome P 450 3A4. Six metabolites with no appreciable vasodilatory effects have been identified.

Excretion :

Is excreted almost entirely as metabolites, about70% of a dose being excreted in urine and the remainder in faeces.

Dose :

Tablet oral - 2.5mg,5mg & 10mg.

Usual Adult Dose for Hypertension:

1. Initial dose : 5 mg daily, adjusted as required.
2. Maintenance dose : 2.5 mg to 10 mg daily and doses above 20mg daily not usually needed.

Usual Adult Dose for Angina:

Initial dose: 5mg daily increased if necessary to 10mg daily.

Adverse drug reactions:

Serious:

Arrhythmias, MI, AV block, palpitations, hypotension, tachycardia, peripheral edema.

Others:

Headache, drowsiness, dizziness, anxiety, diarrhea, gingival hyperplasia, facial edema, pharyngitis.

Drug interactions:

The barbiturate, amobarbital, aprobarbital, butabarbital, butalbital butethal, heptabarbital,hexobarbital,methylphenobarbitalmethohexital,pentobarbital,primidone,quinidine barbiturate secobarbital, talbutal.

Contraindications:

Hypersensitivity, Severe hepatic impairment, Cirrhosis or Biliary obstruction.Primary hyper aldosteronism**,** Pregnancy, Lactation.

International brand names available in the market:

Plendil, Felogard, Renedil.

Storage:

Store in airtight containers, Protect from light.

(Martindale 36th Edition ,Clarke's Analysis of Drugs and Poisons 3rd Edition & Drugbank.com).

CHAPTER VIII

EXCIPIENTS PROFILE

CHAPTER VIII

EXCIPIENT PROFILE

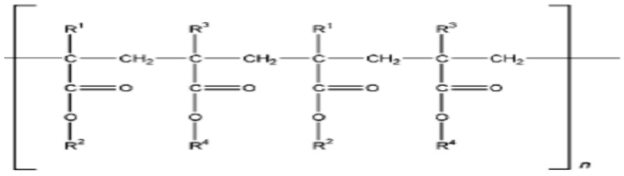
Eudragit L 100 (Polymethacrylate):

Synonyms : Acryl-EZE; Acryl-EZE MP; Eastacryl 30D; Eudragit KollicoatMAE 30 D; Kollicoat MAE 30 DP; Polymeric methacrylates.

Nonproprietary names : Bp: Methacrylic acid–ethyl acrylate copolymer (1: 1) PhEur: Acidum methacrylicum et ethylis acrylas polymerisatum (1: 1). Acidum methacrylicum et ethylis acrylas polymerisatum (1: 1) dispersion 30 per centum. Acidum methacrylicum et methylis methacrylas (1: 1).Acidum methacrylicum et methylis methacrylas Polymerisatum (1: 2).Copolymerum methacrylatis butylati basicum Polyacrylatis dispersion 30 per centum.USPNF: Ammonio methacrylate copolymer, Methacrylic acid copolymer, Methacrylic acid copolymer dispersion.

Chemical name : Poly(methacrylic acid, methyl methacrylate) 1 : 1

Chemical structure:



$R^1, R^3, R^4 = CH3,\ R^2 = H$

Description:

1. Nature : White, free flowing powder.
2. Polymer dry weight content : 95%
3. Recommended solvents : acetone, alcohols.
4. Acid value : 300 – 330.
5. Density : 0.831 – 0.852g/cm^3

Functional category:

1. Film former
2. Tablet binder
3. Tablet diluent

Stability and storage conditions:

Dry powders are stable for a period of 3 years if stored in a tightly closed container at less than 30^o C. Dispersions should be stored at temperatures between 5 ^{o}C and 25 ^{o}C and are stable for a period of 18 months.

(Raymond C Rowe et al., 2009 page no: 525-533).

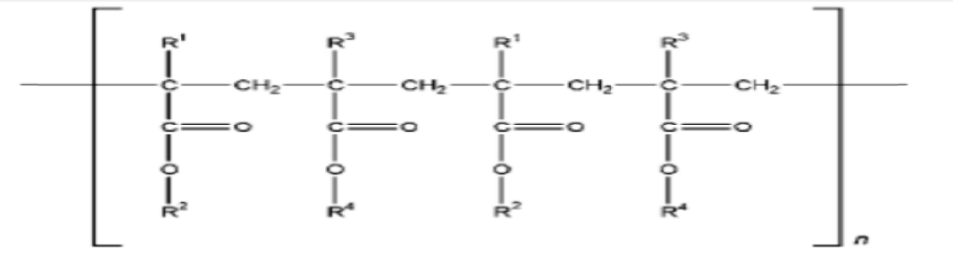
Eudragit S 100 (Polymethacrylate):

Synonyms : Acryl-EZE; Acryl-EZE MP; Eastacryl 30D; Eudragit KollicoatMAE 30 D; Kollicoat MAE 30 DP; Polymeric methacrylates.

Nonproprietary names : Bp: Methacrylic acid–ethyl acrylate copolymer (1: 1) PhEur: Acidum methacrylicum et ethylis acrylas polymerisatum (1: 1) Acidum methacrylicum et ethylis acrylas polymerisatum(1: 1) dispersion 30 per centum Acidum methacrylicum et methylis methacrylas (1: 1) Acidum methacrylicum et methylis methacrylas Polymerisatum (1: 2) Copolymerum methacrylatis butylati basicum Polyacrylatis dispersion 30 per centum USPNF: Ammonio methacrylate copolymer, Methacrylic acid copolymer Methacrylic acid copolymer dispersion.

Chemical name : Poly(methacrylic acid, methyl methacrylate) 1 : 2

Chemical structure:



$R^1, R^3, R^4 = CH3, R^2 = H$

Description:

1. Nature : White, free flowing powder.
2. Polymer dry weight content : 95%
3. Recommended solvents : acetone, alcohols.
4. Acid value : 180 -200.
5. Density : 0.831 – 0.852g/cm^3.

Functional category:

1. Film former
2. Tablet binder
3. Tablet diluent

Stability and storage conditions:

Dry powders are stable for a period of 3 years if stored in a tightly closed container at less than 30^o C. Dispersions should be stored at temperatures between 5 ^{o}C and 25 ^{o}C and are stable for a period of 18 months.

(Raymond C Rowe et al., 2009 page no:525-533).

Poloxamer 188:

Synonyms **:** Pluronic F68; Polyethylene-polypropylene glycol; Poly(Ethylene oxide-co-Polypropylene oxide), Block; Block Copolymer of Ethylene Oxide; Polyoxyethylene-Polyoxypropylene Block Copolymer; Propylene Oxide, Lutrol.

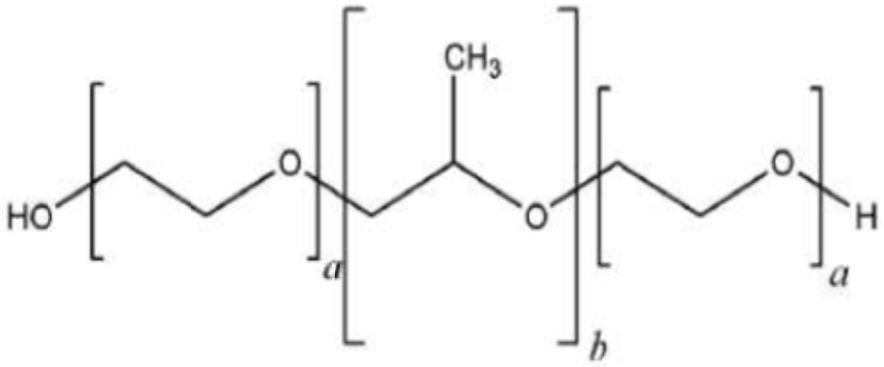
Nonproprietary Names **:** BP : Poloxamers

PhEur : Poloxamera

USPNF: Poloxamer

Chemical Name **:** α-Hydro-ω-hydroxypoly (oxyethylene) poly (oxypropylene) poly(oxyethylene) block copolymer.

Chemical Structure:



Description:

Nature	:	Waxy, free-flowing prilled granules
Color	:	White
Odour	:	Odourless
Taste	:	Tasteless

HLB value	:	29
Melting point	:	52^{o} C - 57^{o}C.
Solubility	:	Freely soluble in 95% ethanol, water

Functional Category :

1. Dispersing agent
2. Coemulsifying agent;
3. Solubilizing agent
4. Tablet lubricant
5. Wetting agent.

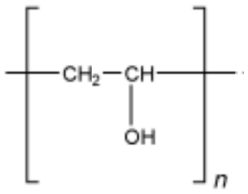
Storage :

Should be stored in a well-closed container in a cool and dry place.

(Raymond C Rowe et al., 2009 page no:506-509).

Polyvinyl Alcohol:

Synonyms	**:**	Airvol
		Alcotex
		Elvanol
		Gelvatol
		Gohesenol
		Lemol
		Mowiol
		Polyvinol
		PVA
		Vinyl alcohol polymer
Nonproprietary Names	**:**	PhEur: Poly(vinylis acetas)
		USP: Polyvinyl alcohol
Chemical Name	**:**	Ethenol
Chemical Formula	**:**	$(C_2H_4O)_n$
Chemical Structure	**:**	



Description:

Nature	:	White to cream-colored granular powder.
Odour	:	Odourless.
Solubility	:	Soluble in water; Slightly soluble in ethanol(95%); Insoluble in organic solvents.

Melting point	:	228^0C for fully hydrolyzed grades; 180-190^0C for partially hydrolyzed grades.
Specific gravity	:	1.19-1.31 for solid at 25^0C.
Specific heat	:	1.67 J/g (0.4 cal/g)

Functional Category:

1. Coating agent
2. Lubricant
3. Stabilizing agent
4. Viscosity-increasing agent

Stability and storage conditions:

Polyvinyl alcohol is stable when stored in a tightly sealed container in a cool, dry place. Aqueous solutions are stable in corrosion-resistant containers. Preservatives may be added to the solution if extended storage is required. Polyvinyl alcohol undergoes slow degradation at 100^0C and rapid degradation at 200^0C; it is stable on exposure to light.(Raymond C Rowe et al., 2009 page no: 564-565).

CHAPTER IX

EXPERIMENTAL PROTOCOL

CHAPTER - IX

EXPERIMENTAL PROTOCOL

1. PREPARATION AND STANDARD CURVES FOR FELODIPINE

a. Preparation of calibration medium

0.1N Hydrochloric Acid with 0.1% w/v SLS

A known volume of 8.5ml Hydrochloric acid is dissolved in distilled water and add 1 gm Sodium lauryl sulphate the volume is made up to 1000ml. (IndianPharmacopoeia., 2010).

Phosphate Buffer pH 6.5 with 0.1% w/v SLS

A known volume of 100ml of phosphate buffer pH 6.5 with 0.1 percent sodium lauryl sulphate prepared by diluting 41.2ml of 1M monobasic sodium phosphate monohydrate, 39.2 ml of 0.5M dibasic sodium phosphate anhydrous, and 1 gm of sodium lauryl sulphate to a 1000ml volumetric flask. Add 900ml of distilled water, adjust with 1M sodium hydroxide to volume with distilled water. (IndianPharmacopoeia., 2010 & U S Pharmacopoeia., 2007).

Phosphate buffer saline (PBS) pH 7.4 with 0.1% SLS

A known quantity of (2.38 g) of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8 g of sodium chloride are dissolved in sufficient quantity of distilled water and made upto 1000ml.

b. Estimation of absorption maximum (λmax)

A known weight (10mg) of drug (Felodipine) is dissolved in 100ml of methanol to form a primary stock solution (100µg/ml). The stock solution is further diluted with acid buffer 0.1N Hydrochloric acid with 0.1% w/v SLS and phosphate

buffer pH 6.5 with 0.1% w/v SLS solution separately to make (10µg/ml) concentration. The resultant solutions is scanned in the range of (200-400nm) by UV Spectrophotometer to get absorption maximum(λ max).

c. Preparation of standard curves

The primary stock solution is further diluted with 0.1N Hydrochloric acid with 0.1% w/v SLS, to obtain a series of solution in the concentration range of (5 to 25µg/ml). The absorbance of the solutions are measured at λmax (362nm) by UV-spectrophotometer. A standard curve is plotted using concentration on X-axis and the absorbance obtained on Y-axis. Similarly, a standard curve is also plotted using pH 6.5 with 0.1% w/v SLS buffer solution.(Sandeep Kumar D et al., 2011, Patel V P et al 2011 & Hariprasanna R C et al., 2010).

2. DRUG POYMER INTERACTION STUDIES

Compatibility studies

Fourier Transform Infrared Spectroscopy **(**FT-IR) are carried out to check the compatibility between the drug and the polymer.

a. Fourier Transform Infrared Spectroscopic analysis (FT-IR)

Fourier Transform Infrared Spectrum analysis of drug (Felodipine), polymers (Eudragit L & S 100) surfactants(Pluronic F 68 & Polyvinyl Alcohol) are obtained from FT-IR Spectrophotometer (Shimadzu,Japan) by KBr disk method. It is an important tool plays a major role in determining the compatibility between the drug and polymer. A known weight of samples are mixed with KBr powder and compressed to 10-mm discs by hydraulic press at pressure of 150 bar for 30 s. The scanning range and resolution are 400 – 4000cm^{-1} and 4cm^{1}.The spectra obtained are compared and interpreted for the shifting of functional peaks or the appearance and disappearance of new functional peaks.(Mishra B et al., 2010).

3. FORMULATION OF FELODIPINE LOADED POLYMERIC NANOPARTICLES

The preparation of felodipine loaded polymeric nanoparticles are prepared according to Solvent displacement / Nanoprecipitation technique.(Bivash Mandal et al., 2010, Suganeswari M et al., 2011 & Srinivas P et al., 2012).

A known weight of felodipine 10 mg, polymers eudragit L 100, eudragit S 100 of different ratios (1:10,1:20,1:30,1:40,1:50,1:60&1:70)) are dissolved well in 10 ml of methanol, forms an organic phase. An aqueous phase which comprises of 20ml of water containing different concentrations of stabilizers 1% pluronic F 68 and 1% polyvinyl alcohol. The organic phase is slowly injected on to the aqueous phase under continuous stirring. To achieve nanoprecipitation, an organic solution of the polymer is simply added to a non- solvent of the polymer (generally water) with which the organic solvent is miscible. Nanoparticles form instantaneously by precipitation of the polymer in narrow window of composition, after which the organic solvent is removed by evaporation under continuous stirring for 3- 4 hours. The plain (drug free) nanoparticles are prepared using the same procedure by omitting the drug.(Nepolean R et al., 2012).

4. CHARACTERIZATION OF FELODIPINE POLYMERIC NANOPARTICLES

The prepared felodipine polymeric nanoparticles are evaluated in terms of drug content, entrapment efficiency, particle size and polydispersity index, zetapotential measurement, *in vitro* drug release studies and kinetics of drug release.

a. Determination of drug content

The total amount of drug content present in the prepared formulation is evaluated using UV- spectrophotometric analysis**.** A known weight 1 mg equivalent

of felodipine is taken from the polymeric nanosuspension which is dissolved in 1ml of methanol and is suitably diluted to 100 ml to make (10µg/ml) concentration using the buffer solution. The absorbance of the above prepared solution is determined at 362nm UV – visible spectrometer. The total drug content can be calculated from the standard curve using the formula given below,

$$\text{Drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

(Sanjay singh et al., 2009).

b. Determination of Entrapment efficiency

The amount of felodipine encapsulated in the polymeric nanoparticles (entrapped drug) are separated from the aqueous medium by ultracentrifugation (Eppendorf Centrifuge, 5417R, Germany) at 14,000 rpm for 90 min at 4˚C. Then, the sample is taken from the separated supernatant liquid and diluted suitably using the buffer solution and the amount of free drug present is determined by measuring the absorbance at 362nm by UV- spectrophotometer. The amount of Felodipine encapsulated in polymeric nanoparticle formulation is calculated from the difference between the total drug content and the amount of unentraped drug remaining in the aqueous medium. The entrapment efficiency (EE) can be calculated as follows

$$\text{Entrapment efficiency} = \frac{\text{Drug content} - \text{unentraped drug}}{\text{Drug content}} \times 100$$

(Abdul Hasan Sathali A and Gopinath M et al., 2013).

c. Determination of Particle Size and Zeta potential

The particle size, polydispersity index (PI) & zeta potential of felodipine loaded polymeric nanoparticulate suspension are studied by dynamic light scattering

technique using a Zetasizer 3000 (Malvern Instruments, UK). It yields the mean particle diameter (average diameter Z-AVE) and the width of particle size distribution (polydispersity index,PI)**.** The aqueous nanoparticulate suspension is diluted with distilled water before analysis and the sample is scattered at a fixed angle of 90°**.** Zeta potential is highly useful for the assessment of the physical stability of colloidal dispersions. The particle surface charge is quantified as zeta potential**.**(Rezaei Mokarram A et al., 2010 & Srinivas P et al., 2012).

d. *in vitro* drug release studies

The *in vitro* drug release studies of felodipine loaded polymeric nansuspension is evaluated using dialysis bag method by diffusion mechanism. The nanoparticulate suspension equivalent to 1 mg of felodipine is placed in a dialysis bag and sealed at both the ends. The dialysis bag which contains the formulation which acts as the donor compartment and a beaker containing the buffer solution acts as a receptor compartment is stirred at a minimum speed and maintained at 37˚C±2˚C.

Initially, the dialysis bag is kept in 0.1N hydrochloric acid with 0.1%w/v SLS acid buffer for a period of 2 hours and then it is placed at pH 6.5 with 0.1%w/v SLS phosphate buffer for the next 10 hours. Aliquots of samples 5ml are withdrawn at subsequent time intervals and the same amount must be replaced on to the diffusion medium with the buffer solution to maintain the sink condition. The samples are withdrawn at an interval of 15 minutes for the first 2 hours and 30 minutes interval for the next10 hours. Finally, the amount of felodipine dissolved is determined by measuring the absorbance at 362nm by UV- spectrophotometer. And, the experiment is performed in triplicate.(Mishra B et al., 2010 & Umar faruksha A et al., 2013).

e. Kinetics of drug release studies

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles are fitted with various kinetic equation like

1. Zero order(cumulative %release vs time).
2. First order(log % drug remaining vs time).
3. Higuchi's model (cumulative %drug release vs square root of time).
4. Hixon-Crowell cube root law.
5. Korsmeyer-peppas model.

1. The zero order equation describes the systems where the drug release rate is independent of its concentration.

$$\mathbf{C = k_0 t}$$

Where C is the concentration of the drug at time (t) and k_0 is the zero-order release rate constant.

2. The first order Equation describes the release from a system where the release rate is concentration dependent.

$$\mathbf{\log C = \log C_0 - kt / 2.303}$$

Where C is the concentration of the drug at time (t), C_0 is the initial concentration of the drug and k is the first-order release rate constant.

3. Higuchi described the release of drugs from porous, insoluble matrix as a square root of time dependent process based on Fickian diffusion as shown below

$$\mathbf{Q = kt^{1/2}}$$

Where Q is the amount of drug released in time t.

4. The Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of particles.

$$\mathbf{W_0^{1/3} - W_t^{1/3} = \kappa t}$$

Where W_0 is the initial amount of drug in the pharmaceutical dosage form, Wt is the remaining amount of drug in the pharmaceutical dosage form at time t, and κ is the constant incorporating the surface-volume relationship.

5. Korsmeyer – Peppas model describes the fraction of drug release relates exponentially with respect to time.

$$\mathbf{M_t/M_\infty = Kt^n}$$

Where M_t/M_∞ is a fraction of drug released at timt t, k is the release rate constant and n is the release exponent.(Abdul Hasan Sathali A and Gopinath M et al., 2013 & Bivash Mandal et al., 2010).

f. Selection and evaluation of best formulation

The best formulations are selected based on the results obtained from particle size analysis, entrapment efficiency, *in vitro* drug release studies and drug release kinetics.

i. Lyophilization of nanosuspensions

Felodipine nanosuspensions are lyophilized by using freeze dryer (Lyodel-Delvac Pumps Pvt. Ltd, USA) to enhance the chemical stability of nanosuspension. The freshly prepared nanosuspensions are lyophilized with cryoprotective agent (mannitol). Briefly, Felodipine nanosuspensions are rapidly cooled down to -50^0C for 2 hours followed by primary drying at I.03 mbar and secondary drying at 0.001 mbar.

Entrapment efficiency and *In vitro* drug release of the lyophilized polymeric nanoparticles (F7 EL 100 with 1% pluronic F68) is then compared with that of pure drug (Felodipine). (Rainer H Muller et al., 2008).

ii. Infrared spectroscopic analysis

Infrared spectrum(IR) analysis is carried out for the selected best formulation to find out the interactions between the drug and excipients used as per the procedure mentioned on drug polymer interaction studies 2(a)**.**

iii. Solubility measurement studies

The solubility of the best formulations of felodipine loaded polymeric nanoparticulate suspension and pure drug in distilled water and phosphate buffer pH 6.5 with 0.1% w/v SLS is carried out in a mechanical shaker apparatus. A known weight equivalent to 1mg of felodipine pure drug and nanosuspension are separately introduced in to the 250 ml stoppered conical flasks containing 10 ml of the respective solvents. The sealed flasks are agitated on a mechanical shaker for 24 hrs. An aliquot is filtered and the filterate is suitably diluted and analysed on a UV-spectrophotometer.(Rezaei Mokarram A et al., 2010 & Kathleen Dillen et al., 2006).

iv. Morphology of Polymeric Nanoparticles by SEM analysis

The morphology of the felodipine polymeric nanoparticulate suspension is studied using scanning electron microscopy (SEM) (S-4800, Hitachi Technologies Corporation, Japan). Prior to examination, the sample is mounted onto metal stubs using a double sided adhesive tape and sputtered with a thin layer of gold under vaccum. The scanning electron microscope is operated at an acceleration voltage of 1.5kv.(Yonggang Yang et al., 2011).

v. *Ex vivo* intestinal permeability studies

The experiments are carried out rat intestinal tissue is used to determine the drug permeation profile.

The three intestinal segments, duodenum, jejunum and ileum using felodipine loaded polymeric nanoparticles for each segments. Rats, fasting for 18 – 20 hours is

anaesthetized by some ether sprinkled to a piece of cotton wool in a glass container equipped with a lid. After making a midline incision in the abdomen, the intestinal segments is isolated as follows: duodenum segment of 8 cm is isolated starting from the pylorus; jejunum segment of 10 – 15 cm is isolated 25 cm from the pylorus; and ileum segments of 10 – 15 cm is isolated 20 cm upwards from caecum.

The isolated segments is washed with phosphate buffer saline (PBS) pH 7.4 to remove any mucous and lumen contents. The contents are removed from segments and again washed with pH 7.4 phosphate buffer. One end of the segment is tied with suture thread and the best formulation selected felodipine loaded polymeric nanosuspension and pure drug (equivalent to 1 mg) are injected separately in three parts of the intestinal segments with the help of syringe and other end of intestine is tied with the help of suture thread. Then the tied intestinal segments is placed in 250 ml beaker containing 100 ml of phosphate buffer pH 7.4 with 0.1% SLS continuously bubbled with 95% O_2 and 5% CO_2 with constant stirring at 50 rpm. The temperature of medium maintained at 37°C± 2°C.

The studies are completed in triplicate manner and aliquots sample of (5 ml) withdrawn at the intervals of 15, 30, 60, 90 and 120 minutes. The fresh phosphate buffer is replaced after every withdrawal to maintain the sink condition. The collected samples is analysed by UV Visible spectophotometrer (Shimadzu UV-1700 pharma spec, Japan) at 362nm. The cumulative amount of drug permeated is plotted against time.(Abdul Hasan Sathali A and Nisha N et al., 2013 & Hadi Valizadeh et al., 2013).

CHAPTER X

RESULTS AND DISCUSSION

TABLES & FIGURES

CHAPTER-X

RESULTS AND DISCUSSION

1. PREPARATION AND STANDARD CURVES FOR FELODIPINE

a. Preparation of calibration medium

The calibration medium of distilled water with 0.1% w/v SLS, 0.1N hydrochloric acid with 0.1% w/v SLS, phosphate buffer pH6.5 with 0.1% w/v SLS and phosphate buffer pH7.4 with 0.1% w/v SLS were prepared as per the Indian pharmacopoeia**.**

b. Estimation of absorption maximum

The absorption maximum (λmax) of felodipine was estimated by scanning the (10µg/ml) concentration of the drug solution in UV- region (200- 400nm). The obtained spectrum showed the λmax of 362nm in distilled water with 0.1% w/v SLS, 0.1N hydrochloric acid with 0.1% w/v SLS, phosphate buffer pH 6.5 with 0.1% w/v SLS and phosphate buffer pH7.4 which was shown in the **Figure. (20a-20d).**

c. Preparation of standard curves of Felodipine

The standard curves of felodipine were prepared using distilled water, 0.1N hydrochloric acid with 0.1% w/v SLS, phosphate buffer pH 6.5 with 0.1% w/v SLS. The absorbance of the solutions (5-25µg/ml) was measured in UV-Spectrophotometer at 362nm. The linear correlation coefficient obtained was same for both 0.1N hydrochloric acid with 0.1% w/v SLS and phosphate buffer pH6.5 with 0.1% SLS, and it was found to be $\gamma = 0.999$. Felodipine obeys the Beer's law within the concentration range of (5-25µg/ml). The standard curves of Felodipine were shown in

Table(1A-1D) and Figure(20e-20h).(Sandeep Kumar D et al., 2011, Patel V P et al., 2011, Hariprasanna R C et al., 2010, Raghavendra Rao N G et al., 2010).

2. DRUG –POLYMER INTERACTION STUDIES

a. Infrared spectroscopic analysis

Infrared spectroscopic analysis were carried out to confirm the compatibility between drug and the polymers used for the preparation of polymeric nanoparticles. The infrared studies were performed for pure drug, polymers and physical mixture of drug and polymers. The spectra studied at 4000cm^{-1} to 400 cm^{-1} were shown in (**Figure 21a-21i)**.

The IR spectrum of pure drug obtained was compared with the spectra of physical mixtures of drug and the polymers showed that there was no shifting of functional peaks.

S.NO	CHARACTERISTICS	WAVE NUMBER(cm^{-1})
1	N-H stretching	3095.85 cm^{-1}
2	C-H stretching in CH_2	2963.93 cm^{-1}
3	C-H stretching	2902.96 cm-1
4	C=O stretching	1696.45 cm-^{1}
5	C=C stretching	1662.69 cm-1
6	C-N stretching	1363.72 cm^{-1}
7	C-C stretching	1172.76 cm-1
8	C-OCH_3 stretching	1058.96 cm-1
9	C-Cl	705.01 cm^{-1}

All the major peaks present in the spectrum of pure drug were clearly observed in the spectrum of physical mixtures with negligible changes. From the obtained results, it was concluded that there was no interactions between the drug and excipients.

3. FORMULATION OF FELODIPINE LOADED POLYMERIC NANOPARTICLES

Polymeric nanoparticles were prepared using eudragit L100 and eudragit S100 by nanoprecipitation technique. This method developed by Fessi et al.,1992. represents an easy and reproducible technique. This method is based on the interfacial deposition of a polymer following displacement of a semi polar solvent miscible with water from a lipophilic solution.(Bivash Mandal et al., 2010, Ugo Bilati et al., 2005 & Snjezana Stolnik et al.,1999).

All the formulations of felodipine (F1-F28) were prepared using polymers (Eudragit L100 & Eudragit S100) at different ratios (1:10,1:20,1:30,1:40,1:50,1:60 & 1:70) and stabilizers (Pluronic F68 and Polyvinyl alcohol) at concentration of 1%. The different polymer ratios and surfactant concentrations were shown in the **Table(2A-AD).**(Swarnali Das et al.,2010).

The polymeric nanoparticles were prepared by dissolving the polymers (Eudragit L100 and Eudragit S100) using an organic solvent (methanol), with or without felodipine and the organic phase was introduced drop wise into the stirred surfactant aqueous phase containing concentration of 1% pluronic F68 and 1% polyvinyl alcohol resulting in a colloidal suspension. Instantaneous formation of a colloidal suspension occurred as a result of the polymer deposition on the interface between the organic phase and water, when partially water miscible organic solvent

(methanol) diffused out quickly into the aqueous phase from each transient particle intermediate.(Sanjay Singh et al.,2009, Srinivas et al.,2012 & Nepolean R et al.,2012).

4. CHARACTERIZATION OF FELODIPINE LOADED POLYMERIC NANOPARTICLES

a. Determination of drug content

The drug content for all the prepared formulations (F1 to F28) were found to be in the range of 91.08% to 94.82% which was shown in **Table(3A-3D).** The results indicated that there was uniform distribution of drug in all nanoparticle formulations. (Sanjay Singh et al., 2009).

b. Determination of entrapment efficiency

Polymeric nanoparticle formulations, the impact of polymer and stabilizer concentration on entrapment efficiency was considerably significant. The ranges of entrapment efficiency of formulations F1-F28 were 29.72% to 63.95% as shown in **Table(4A-4D) and Figure(22)**.(Sanjay Singh et al., 2009).

The formulations F1-F28, the maximum entrapment efficiency was obtained for the formulation F7 eudragit L100 with 1% pluronic F68 was found to be 63.95% respectively. It could be attributed to the higher concentration of polymer (1:70) ratio in the nanoparticle formulation. The fabrication parameters such as different drug-polymer ratios and different stabilizers at concentrations were used to achieve the highest entrapment of felodipine.

i. Influence of drug-polymer ratio

The entrapment efficiency of felodipine loaded eudragit L100 and eudragit S100 nanoparticles was greatly influenced by the drug- polymer ratios. The results were shown in **Table(4A-4D) and Figure(22)**.(Peng et al., 2007).

Formulations F1-F7 prepared using different ratios of polymer (Eudragit L100) (1:10,1:20,1:30,1:40,1:50,1:60&1:70) containing 1% pluronic F68 as stabilizer showed the entrapment efficiency of **29.72%, 30.05%, 31.90%, 33.65%, 45.66%, 57.86% & 63.95%** respectively. The entrapment efficiency was increased in the order of **F2<F1<F3<F4<F5<F6<F7.**

Formulations F8-F14 prepared using different ratios of polymer (Eudragit L100) (1:10,1:20,1:30,1:40,1:50,1:60&1:70) containing 1% polyvinyl alcohol as stabilizer showed the entrapment efficiency of **30.81%, 31.46%, 34.99%, 39.42%, 43.37%, 51.07% & 59.38%** respectively. The entrapment efficiency was increased in the order of **F8<F9<F10< F11<F12<F13<F14.**

Formulations F15-F21 prepared using different ratios of polymer (Eudragit S100) (1:10,1:20,1:30,1:40,1:50,1:60&1:70) containing 1% pluronic F68 as stabilizer showed the entrapment efficiency of **31.55%, 32.02%, 33.05%, 35.75%, 44.77%, 50.22% & 58.02%** respectively. The entrapment efficiency was increased in the order of **F15<F16< F17<F18<F19<F20<F21.**

Formulations F22-F28 prepared using different ratios of polymer (Eudragit S100) (1:10,1:20,1:30,1:40,1:50,1:60&1:70) containing 1% polyvinyl alcohol as stabilizer showed the entrapment efficiency of **30.45%, 31.90%, 34.12%, 34.28%, 40.28%, 46.02% & 53.29%** respectively. The entrapment efficiency was increased in the order of **F22<F23<F24<F25<F26<F27<F28.**

From the results it was observed the felodipine nanoparticle formulations prepared with 1:70 ratio of eudragit L100 showed the higher entrapment than the other formulations prepared with (1:10,1:20,1:30,1:40,1:50,&1:60) ratios of eudragit L100. The high entrapment efficiency of felodipine was believed to be due to its poor

aqueous solubility, high affinity of drug and polymer affinity in the same organic solvent (methanol) and increased polymer ratio. This is increase in polymer concentration in organic phase enhanced the drug entrapment due to increase in organic phase viscosity, which increased the diffusional resistance to drug molecules from organic phase to aqueous phase, thereby entrapping more drug in the polymeric nanoparticles.(Poovi G et al.,2011, Swarnali Das et al., 2010 & Annick Ludwig et al., 2006).

ii. Influence of stabilizer concentration

The effect of stabilizers (Pluronic F68 & Polyvinyl alcohol) on the entrapment efficiency of felodipine loaded eudragit L100 and eudragit S100 nanoparticles were investigated. The results were shown in the **Table(4A-4D) and Figure(22)**. Felodipine loaded polymeric nanoparticles prepared with pluronic F68 showed better entrapment than those prepared with polyvinyl alcohol.

Felodipine loaded polymeric nanoparticles prepared with pluronic F68 showed better entrapment than those prepared with polyvinyl alcohol.(Anilkumar J Shinde et al., 2011).

c. Determination of particle size, polydispersity index & zeta potential

The particle size diameter(Z-AVE), polydispersity index(PI) and zeta potential of the prepared nanoparticulate suspension were studied by dynamic light scattering microscopy.(Annick Ludwig et al.,2006).

The particle size in the range of less than 400nm were preferred in pharmaceutical process development. The mean particle diameters of high entrapment efficiency of the formulations (F7,F14,F21&F28) was found to be in the range of 192.4nm, 238.7nm, 210.3nm & 298.4nm as shown in **Table(5A&5B) & (Figure23a-**

23d). The formulations in terms of drug / polymer ratios, surfactant concentration as well as the volume of external phase resulted in significant differences in particle size.(Jawahar N et al.,2009).

i). Influence of drug –polymer ratio on particle size:

The influence of drug- polymer ratio (1:70) of felodipine loaded eudragit L100 and eudragit S100 nanoparticles on the particle size was investigated. The results were shown in **Table.(5A&5B)&(Figure23a-23d).**

Formulations F7, F14 prepared using polymer (Eudragit L 100) (1:70) containing 1% pluronic F68 & 1% polyvinyl alcohol as stabilizer showed the particle size of **192.4nm,238.7nm** respectively The mean particle size was increased in the order of **F7 < F14.**

Formulations F21, F28 prepared using polymer (Eudragit S 100) (1:70) containing 1% pluronic F68 & 1% polyvinyl alcohol as stabilizer showed the particle size of **210.3nm&298.4nm** respectively The mean particle size was increased in the order of **F21 < F28.**

It was concluded that an increase in polymer concentration increased the particle size of nanoparticles. i.e., the particle size of felodipine loaded polymeric nanoparticles showed a positive relationship with (Eudragit L100 & Eudragit S100) concentration.

This is because, increasing polymer concentration led to increase in the viscosity of the organic phase. i.e., high viscous resistance to the shear forces hinder the nanoparticle formation. A more viscous organic phase provides a higher mass transfer resistance, the diffusion of polymer – solvent phase into the external aqueous phase is reduced and larger nanoparticles are formed.(Annick Ludwig et al.,2006).

ii). Influence of stabilizers on particle size

Felodipine loaded polymeric nanoparticles were prepared using two different stabilizers (Pluronic F68 & Polyvinyl alcohol) at 1% concentration as shown in **Table.(5A&5B)&(Figure23a-23d).**

Formulation F7 prepared with 1% pluronic F68 showed the mean particle size of 192.4nm respectively.

Formulation F14 prepared with 1% polyvinyl alcohol showed the mean particle size of 238.7nm respectively.

Formulation F21 prepared with 1% pluronic F68 showed the mean particle size of 210.3nm respectively.

Formulation F28 prepared with 1% polyvinyl alcohol showed the mean particle size of 298.4nm respectively.

Thus it was concluded that, at high concentration, more amount of stabilizers could be oriented at organic solvent/water interface to reduce efficiently the interfacial tension, which resulted in significant increase in the net shear stress at a constant energy density during emulsification and promoted the formation of smaller emulsion droplets (Anilkumar J Shinde et al., 2010).

Stabilization of nanoparticles in polymeric nanosuspension requires a stabilizer that binds on to the particle surface. Pluronic F68 adsorb strongly on to the surface of hydrophobic nanoparticles via their hydrophobic polyoxypropylene centre block and have been shown to be quite successful in regard to stabilization of nanoparticles.

Formulations prepared with pluronic F68 (F7 EL 100 & F21 ES 100) showed the particle size in the 192.4nm & 210.3nm. Similarly, Formulations prepared with polyvinyl alcohol (F14 EL 100 & F28 ES 100) showed the particle size in the 238.7nm & 298.4nm. Two stabilizers, pluronic F68 and polyvinyl alcohol were tried to stabilize the formulation. The average particle size obtained using pluronic F68 was lesser than that obtained with polyvinyl alcohol.(Annick Ludwig et al.,2006 & Jawahar N et al.,2009).

Polydispersity Index

Polydispersity Index (PI), plays an important role in the physical stability of nanosuspension and should be as low as possible for long term stability. The polydispersity index of high entrapment efficiency of the formulations (F7, F14, F21 & F28)) were shown in the **Table.(5A&5B)&(Figure23a-23d).** It is a measure of dispersion homogeneity and ranges from 0 to 1.Values close to 0 indicates a homogenous dispersion while those greater than 0.3 indicate high heterogeneity.

The polydispersity index of formulations (F7&F14) composed of EL 100 and ES 100 at concentrations 1% pluronic F68 and 1% polyvinyl alcohol was found to be in the range of 0.132 & 0.165.

The polydispersity index of formulations (F21&F28) composed of EL 100 and ES 100 at concentrations 1% pluronic F68 and 1% polyvinyl alcohol was found to be in the range of 0.159 & 0.172.

All of them exhibited a PI value of less than 0.3,which showed a relative homogenous dispersion.(Kristl B et al., 2002).

Zetapotential

Colloidal stability of the prepared formulations were measured in terms of Zeta potential. Zeta potential of felodipine loaded polymeric nanoparticulate suspensions were investigated to study the effect of different polymers and stabilizers on surface charge of nanoparticles. The results were shown in the **Table(6A-6B) and Figure(24a-24d)**.

Zeta potential of formulations F7, F14 prepared with EL 100 showed negative zeta potential (-19.4mV& -12.1mV).

Zeta potential of formulations F21, F28 prepared with ES 100 showed negative zeta potential (-16.2mV & -10.9mV).

The zeta potential of the nanoparticles were found to be negative due to the presence of terminal carboxylic groups of polymers (Eudragit L100 & Eudragit S100). High potential values should be achieved in order to ensure a high energy barrier and favour a good stability. According to Muller, a zeta potential of about -25mV allows an ideal stabilization of nanoparticles because the repulsive forces prevent aggregation upon ageing. For electrostatically stabilized nanosuspension a minimum zeta potential of ± 30mV and for combined steric and electrostatic stabilization it should be a minimum of ±20mV.(Dianrui Zhang et al., 2012 & Philippe Maincent et al.,1998).

d. *Invitro* release studies of Felodipine loaded polymeric nanoparticle using dialysis membrane.

The *in vitro* release of felodipine from the polymeric nanoparticles was studied in vitro by the dialysis bag diffusion technique. Initially , the release studies was performed at 0.1N hydrochloric acid with 0.1% w/v of SLS for the first 2 hours,

and at Phosphate buffer pH6.5 with 0.1% w/v of SLS for the next 10 hours to mimic the *in vivo* condition , since the aim of this study was to administer the nanoparticles by the oral route. The dialysis bag retained nanoparticles and allow the diffusion of the drug immediately into the receptor compartment.

The formulations F1 to F7 prepared using different concentrations (1:10, 1:20, 1:30, 1:40, 1:50,1:60 & 1:70) of Eudragit L100 with (1%) pluronic F68 exhibited an initial burst release of **19.67%, 18.03%, 18.91%, 18.42%, 18.50%, 16.01%, 15.71%** at 2hrs & **72.69%, 69.30%, 67.40%, 65.65%, 63.01%, 59.69%, 56.12%** at 12hrs respectively. The cumulative % drug release was decreased in the order of **F1 > F2> F2 > F2>F5>F6>F7**.

The formulations F8 to F14 prepared using different concentrations (1:10, 1:20, 1:30, 1:40, 1:50, 1:60 & 1:70) of Eudragit L100 with 1% Polyvinyl alcohol exhibited an initial burst release of **21.40%, 20.33%, 18.92%, 18.46%, 17.25%, 16.66%, 15.73%** at 2hrs & **74.54%, 72.50%, 68.81%, 66.59%, 63.29%, 60.53%, 57.36%** at 12hrs respectively. The cumulative % percentage drug release was decreased in the order of **F8 > F9> F10 > F11>F12>F13>F14.**

The formulations F15 to F21 prepared using different concentrations (1:10, 1:20, 1:30, 1:40, 1:50, 1:60 & 1:70) of Eudragit S100 with (1%) Pluronic F68 exhibited an initial burst release of **21.74%, 19.59%, 18.73%, 18.07%, 17.50%, 15.96%, 16.15%** at 2hrs & **75.60%, 73.12%, 70.38%, 66.29%, 65.20%, 60.02%, 58.21%** at 12hrs respectively. The cumulative % drug release was decreased in the order of **F15 > F16> F17 > F18 > F19>F20>F21.**

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The formulations F22 to F28 prepared using different concentrations (1:10, 1:20, 1:30, 1:40, 1:50, 1:60 & 1:70) of Eudragit S100 with (1%) Polyvinyl alcohol exhibited an initial burst release of **21.56%, 21.22%, 20.06%, 18.91%, 18.10%, 16.00%, 15.26%** at 2hrs & **75.62%, 73.52%, 71.80%, 67.52%, 66.46%, 61.85%, 58.55%** at 12hrs respectively. The cumulative % drug release was decreased in the order of **F22 > F23> F24 > F25 > F26>F27>F28.**

The cumulative % drug release for all the prepared formulations (F1-F28) results were shown in the **Table.(7A-7D) & Figure.(25a-25d).** The smaller size nanoparticles prepared with lower amount of Eudragit L100 & Eudragit S100 exhibited higher drug release rate, this might be due to the increased nanoparticle surface resulting in larger drug fraction exposed to the dissolution medium and also the higher amount of drug loading. The larger size nanoparticles prepared with higher amount of Eudragit L100 & Eudragit S100 exhibited lower drug release rates, this might due to the decreased nanoparticle surface resulting in smaller drug fraction exposed to the dissolution medium.(Jawahar N et al.,2009).

A higher burst release was detected with in first two hours for the formulations containing lesser amount of polymer ratio(1:20) as shown in **Table.(7A-7D) & Figure.(25a-25d).** By increasing the polymeric concentration up to (1:70) ratio, the burst release was reduced and a sustained release was obtained for 12 hrs due to the entrapment of drug into polymer matrix in the nanoparticles thus, prolonged the drug release.

Among the formulations F1-F7, the burst release was decreased & increased in the order of **F2>F1>F3>F4>F5>F6>F7.**

Among the formulations F8-F14, the burst release was decreased in the order of **F8>F9>F10>F11>F12>F13>F14.**

Among the formulations F15-F21, the burst release was decreased in the order of **F15>F16>F17>F18>F19>F20>F21.**

Among the formulations F22-F28, the burst release was decreased in the order of **F22>F23>F24>F25>F26>F27>F28.**

Burst phase occurs due to the adsorption of drug in the polymeric surface which was, however followed by hydration of the nano-matrix which brings about an increment in the diffusional path length of molecules and consequently the rate of their diffusion becomes lower. Therefore, gaining of controlled release profile and its maintenance could be assumed to be dependent upon the relative hydration rate of the polymer and integrity of the hydrated matrix. Therefore, superiority of one formulation over the other could be established on the basis of higher entrapment efficiency, avoidance of burst release, achievement of a controlled release profile and its maintenance in a time dependent manner. Among that, F7 Eudragit L100, 1% Pluronic F68 possessing higher entrapment efficiency 63.95% and least burst release profile 15.71% than the rest of the formulations.(Mishra B et al., 2010 & Bivash Mandal et al., 2010).

e. Kinetics of drug release

The results obtained from the *in vitro* release studies were attempted to fit into various mathematical models as follows:

a) Cumulative percentage drug release Vs time (zero order rate kinetics)

b) Log cumulative percentage drug remaining Vs time (first order rate kinetics)

c) Cumulative percentage drug release Vs square root of time (Higuchi classical diffusion model)

d) Cube root of percentage drug remaining Vs time (Hixon Crowell erosion equation).

e) Log cumulative percentage drug release Vs log time (Korsmeyer Peppas exponential equation)

Various plots of zero order, first order, Higuchi matrix, Korsmeyer - Peppas and Hixon – Crowell were shown in **Figure.(26-30).** The regression coefficient (r^2) and n values were shown in **Table.(8A-8D).**

Higuchi describes drug release as a diffusion process based on Fick's law, which is square root of time dependent. The amount of drug release from felodipine loaded polymeric nanoparticle formulations (F1-F28) showed a linear relationship with square root of time. Hence, the drug release rate could be expressed by higuchi diffusion model (r^2=0.976 to 0.988). The high correlation coefficients were obtained for the first order drug release kinetics for all the formulations ranging from (r^2 = 0.975 to 0.991).

Different values of n for cylindrical, spherical and slab of geometrices are available in the literature. For spheres, values of 0.5, 0.5 < n < 1.0, 1.0 and higher than 1.0 are related to Fickian diffusion, anomalous, case II transport and super case II transport respectively. According to the data presented in the tables, the values of exponent n were within 0.5<n which indicated that the drug release mechanism followed non- fickian diffusion.(Abdul Hasan Sathali A and Priyanka et al.,2012, Annick Ludwig et al., 2006 & Prasanthi. B et al., 2012).

f. Selection and evaluation of best formulation

From the above results characterization **F7 EL 100 with 1% Pluronic F68** was selected as the best formulation showing,

Particle size	:	192.4 nm.
Entrapment efficiency	:	63.95%
In vitro drug release	:	56.12% in 12 hours
Release kinetics	:	Closest linearity to first order kinetics.

i. Lyophilization of nanosuspensions

Lyophilization of nanosuspensions to obtain the polymeric nanoparticles.

Determination of entrapment efficiency and *in vitro* release

The entrapment efficiency the formulation (F7 Eudragit L 100 with 1% Pluronic F68) containing pluronic F68 as stabilizer showed higher entrapment efficiency. These might be due lyophilized polymeric nanoparticles in entrapment efficiency in 62.82%. Due to high entrapment, drug release from polymeric nanoparticles was sustained. According to these drug release profile in 55.37% in 12 hours. It was found that cumulative percentage drug release of F7 EL 100 formulation were increased due to particle size reduction as compared to pure drug were shown in the **Table.(7E) & Figure.(25e).**

ii. Infra red spectrum analysis

IR spectrum of the best formulation (F7 EL 100 with 1% Pluronic F68) was recorded and shown in the **Figure.(31)**. Pure drug (Felodipine) showed its characteristic peaks at 3095.85,2963.93,2902.96, 1696.45, 1662.69, 1363.72, 1172.76,

1058.96, 705.01 cm^{-1}. The IR spectrum of pure drug obtained was compared with the spectra of best formulations showed that there was no shifting of functional peaks. All the major peaks present in the spectrum of pure drug were clearly observed in the spectrum of best formulations without any significant changes. From the obtained results, it was concluded that there was no interactions between the drug and the excipients used in the formulation.(Abdul Hasan Sathali A and Priyanka K et al.,2012).

iii. Solubility measurement studies

The solubility measurement study was conducted for the best formulation (F7 EL 100 with 1% Pluronic F68) as well as the pure drug using distilled water and phosphate buffer pH 6.5 with 0.1% SLS as shown in **Table.(9) & Figure.(32a&32b).**

The solubility of formulation F7 EL 100 with 1% pluronic F68 & pure drug in distilled water were 117.23µg/ml and 29.91µg/ml respectively. The solubility of formulation F7 EL 100 with 1% pluronic F68 & pure drug in phosphate buffer pH6.5 with 0.1% SLS were 260.58µg/ml and 43.89µg/ml respectively. It was observed that the best formulations showed highest solubility compared to the pure drug in both distilled water and phosphate buffer pH6.5 with 0.1% SLS were shown in **Table.(9) & Figure.(32a&32b) (**Dianrui Zhang et al., 2012, Arunkumar N et al., 2009 & Abdul Hasan Sathali A and Gopinath M et al.,2013).

iv. Morphology of polymeric nanoparticles by SEM analysis

In order to characterize the morphology of nanoparticle formulations SEM analysis was performed. The SEM Photograph of the selected best formulations F7(Felodipine+Eudragit L100+1% Pluronic F68) were shown in **Figure.(33).**The

results indicated that the nanoparticles are spherical in shape and below 1µm in size. (Julijana Kristl et al.,2009 & Abdul Hasan Sathali A and Priyanka K et al.,2012).

v. *Ex vivo* intestinal permeability studies

The results were shown in **Table.(10A -10C) & Figure.(34a - 34c).**

In the duodenum region of the intestinal segment, the cumulative amount of drug permeated for pure drug solution was about 0.22 mg and the polymeric nanoparticle formulation had the permeability of about 0.85 mg (F7) at the end of 2 hrs.

Similarly in the jejunum region also, the cumulative amount of drug permeated for pure drug solution was 0.25 mg and for the polymeric nanoparticle formulations had the permeability of 0.89 mg (F7) at the end of 2 hrs. The same type of results was also obtained from the ileum region of rat intestine. The pure drug solution had the cumulative amount of drug permeability of 0.23 mg and the solid lipid nanoparticle formulations had the permeability of 0.87 mg (F7) at the end of 2 hrs.The intestinal permeability was found to be increase in the **F7 EL 100 with 1% Pluronic F68 > Pure drug.**

From the results, it was observed that the nanoparticle formulations showed better permeability than the pure drug solution.

(Abdul Hasan Sathali A and Nisha et al.,2013).

TABLE 1A: CALIBRATION CURVE OF FELODIPINE USING DISTILLED WATER WITH 0.1% SLS

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE± SD
1	5	0.080±0.0015
2	10	0.181±0.0015
3	15	0.265±0.0020
4	20	0.361±0.0035
5	25	0.448±0.0026

n =3* **γ = 0.999564**

TABLE 1B: CALIBRATION CURVE OF FELODIPINE USING 0.1N HYDROCHLORIC ACID WITH 0.1% SLS

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE± SD
1	5	0.087±0.0015
2	10	0.186±0.0020
3	15	0.270±0.0025
4	20	0.363±0.0045
5	25	0.456±0.0041

n =3* **γ = 0.999570**

TABLE 1C: CALIBRATION CURVE OF FELODIPINE USING PHOSPHATE BUFFER pH 6.5 WITH 0.1% SLS

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE± SD
1	5	0.086±0.0017
2	10	0.187±0.0015
3	15	0.270±0.0025
4	20	0.364±0.0015
5	25	0.447±0.0026

n =3* **γ = 0.999459**

TABLE 1D: CALIBRATION CURVE OF FELODIPINE USING PHOSPHATE BUFFER pH 7.4 WITH 0.1% SLS

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE± SD
1	5	0.079±0.0015
2	10	0.176±0.0015
3	15	0.269±0.0025
4	20	0.368±0.0026
5	25	0.450±0.0025

n =3* **γ = 0.999576**

TABLE 2A:COMPOSITION OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F68 AND 1% POLYVINYL ALCOHOL AS STABILIZER.

FORMULATION CODE	DRUG:POLYMER RATIO	WEIGHT OF DRUG	EUDRAGIT L 100	CONCENTRATION OF PLURONIC F68	CONCENTRATION OF POLYVINYL ALCOHOL
F1	1:10	10mg	100mg	1%	-
F2	1:20	10mg	200mg	1%	-
F3	1:30	10mg	300mg	1%	-
F4	1:40	10mg	400mg	1%	-
F5	1:50	10mg	500mg	1%	-
F6	1:60	10mg	600mg	1%	-
F7	1:70	10mg	700mg	1%	-

TABLE 2B: COMPOSITION OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AND 1% POLYVINYL ALCOHOL AS STABILIZER.

FORMULATION CODE	DRUG:POLYMER RATIO	WEIGHT OF DRUG	EUDRAGIT L 100	CONCENTRATION OF PLURONIC F68	CONCENTRATION OF POLYVINYL ALCOHOL
F8	1:10	10mg	100mg	-	1%
F9	1:20	10mg	200mg	-	1%
F10	1:30	10mg	300mg	-	1%
F11	1:40	10mg	400mg	-	1%
F12	1:50	10mg	500mg	-	1%
F13	1:60	10mg	600mg	-	1%
F14	1:70	10mg	700mg	-	1%

TABLE 2C: COMPOSITION OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AND 1% POLYVINYL ALCOHOL AS STABILIZER.

FORMULATION CODE	DRUG:POLYMER RATIO	WEIGHT OF DRUG	EUDRAGIT S 100	CONCENTRATION OF PLURONIC F68	CONCENTRATION OF POLYVINYL ALCOHOL
F15	1:10	10mg	100mg	1%	-
F16	1:20	10mg	200mg	1%	-
F17	1:30	10mg	300mg	1%	-
F18	1:40	10mg	400mg	1%	-
F19	1:50	10mg	500mg	1%	-
F20	1:60	10mg	600mg	1%	-
F21	1:70	10mg	700mg	1%	-

TABLE 2D: COMPOSITION OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AND 1% POLYVINYL ALCOHOL AS STABILIZER.

FORMULATION CODE	DRUG:POLYMER RATIO	WEIGHT OF DRUG	EUDRAGIT S 100	CONCENTRATION OF PLURONIC F68	CONCENTRATION OF POLYVINYL ALCOHOL
F22	1:10	10mg	100mg	-	1%
F23	1:20	10mg	200mg	-	1%
F24	1:30	10mg	300mg	-	1%
F25	1:40	10mg	400mg	-	1%
F26	1:50	10mg	500mg	-	1%
F27	1:60	10mg	600mg	-	1%
F28	1:70	10mg	700mg	-	1%

TABLE 3A: DRUG CONTENT OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES USING 1% PLURONIC F 68

S.NO	FORMULATION CODE	DRUG CONTENT (%) ± SD*
1	F1	93.58%±2.14
2	F2	91.08%±0.81
3	F3	92.68%±1.11
4	F4	93.75%±1.63
5	F5	92.50%±1.60
6	F6	93.04%±2.44
7	F7	93.04%±1.60

n=3*

TABLE 3B: DRUG CONTENT OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES USING 1% POLYVINYL ALCOHOL

S.NO	FORMULATION CODE	DRUG CONTENT (%) ± SD*
1	F8	93.04%±1.92
2	F9	92.33%±2.16
3	F10	93.22%±2.16
4	F11	91.26%±1.34
5	F12	91.08%±2.94
6	F13	91.79%±1.63
7	F14	93.04%±2.14

n=3*

TABLE 3C: DRUG CONTENT OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES USING 1% PLURONIC F 68

S.NO	FORMULATION CODE	DRUG CONTENT (%) ± SD*
1	F15	94.82% ±1.11
2	F16	92.86% ±3.47
3	F17	94.82% ±2.69
4	F18	92.15% ±2.22
5	F19	93.22% ±2.41
6	F20	93.04% ±2.44
7	F21	91.26% ±1.11

n=3*

TABLE 3D: DRUG CONTENT OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES USING 1% POLYVINYL ALCOHOL

S.NO	FORMULATION CODE	DRUG CONTENT (%) ± SD*
1	F22	94.82% ±2.16
2	F23	94.29% ±3.48
3	F24	93.93% ±1.72
4	F25	93.58% ±2.14
5	F26	92.51% ±2.14
6	F27	92.15% ±1.71
7	F28	92.15% ±1.71

n=3*

TABLE 4A: ENTRAPMENT EFFICIENCY OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES USING 1% PLURONIC F 68

S.NO	FORMULATION CODE	ENTRAPMENT EFFICIFICENCY(%) ± SD*
1	F1	30.05±3.53
2	F2	29.72±3.27
3	F3	31.90±3.20
4	F4	33.65±3.60
5	F5	45.66±1.05
6	F6	57.86±3.07
7	F7	63.95±3.50

n=3*

TABLE 4B: ENTRAPMENT EFFICIENCY OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES USING 1% POLYVINYL ALCOHOL

S.NO	FORMULATION CODE	ENTRAPMENT EFFICIFICENCY(%) ± SD*
1	F8	30.81±2.88
2	F9	31.46±0.58
3	F10	34.99±0.63
4	F11	39.42±3.31
5	F12	43.37±3.82
6	F13	51.07±3.33
7	F14	59.38±1.34

n=3*

TABLE 4C: ENTRAPMENT EFFICIENCY OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES USING 1% PLURONIC F 68

S.NO	FORMULATION CODE	ENTRAPMENT EFFICIFICENCY(%) ± SD*
1	F15	31.55±3.63
2	F16	32.02±0.82
3	F17	33.05±1.38
4	F18	35.75±2.27
5	F19	44.77±2.07
6	F20	50.22±2.22
7	F21	58.02±2.24

n=3*

TABLE 4D: ENTRAPMENT EFFICIENCY OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES USING 1% POLYVINYL ALCOHOL

S.NO	FORMULATION CODE	ENTRAPMENT EFFICIFICENCY(%) ± SD*
1	F22	30.45±0.15
2	F23	31.90±1.72
3	F24	34.12±2.89
4	F25	34.28±2.48
5	F26	40.48±1.51
6	F27	46.02±2.26
7	F28	54.47±1.24

n=3*

TABLE 5A: PARTICLE SIZE OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F68 & 1% POLYVINYL ALCOHOL AS STABILIZER

S.NO	FORMULATION CODE	PARTICLE SIZE(nm)	POLYDISPERSITY INDEX
1.	F7	192.4	0.132
2.	F14	238.7	0.165

TABLE 5B: PARTICLE SIZE OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F68 & 1% POLYVINYL ALCOHOL AS STABILIZER

S.NO	FORMULATION CODE	PARTICLE SIZE(nm)	POLYDISPERSITY INDEX
1.	F21	210.3	0.159
2.	F28	298.4	0.172

TABLE 6A: ZETA POTENTIAL OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F68 & 1% POLYVINYL ALCOHOL AS STABILIZER

S.NO	FORMULATION CODE	ZETA POTENTIAL(mV)
1.	F7	-19.4
2.	F14	-12.1

TABLE 6B: ZETA POTENTIAL OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F68 & 1% POLYVINYL ALCOHOL AS STABILIZER

S.NO	FORMULATION CODE	ZETA POTENTIAL(mV)
1.	F21	-16.2
2.	F28	-10.9

TABLE 7A: COMPARISON OF *IN VITRO* RELEASE OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING PLURONIC F 68 1% AS STABILIZER

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE						
		F1 EL 100	F2 EL 100	F3 EL 100	F4 EL 100	F5 EL 100	F6 EL 100	F7 EL 100
1.2	0.25	5.17±0.83	5.17±0.83	4.99±0.54	4.99±0.54	4.99±0.54	4.99±0.54	4.62±0.31
	0.50	6.52±0.81	7.61±0.86	6.69±0.85	6.51±1.15	6.51±0.86	5.97±0.86	6.31±0.56
	0.75	9.20±1.16	9.44±1.16	8.84±1.69	9.74±1.19	9.19±1.44	7.71±1.51	8.80±1.13
	1.00	12.73±0.91	11.88±0.94	12.35±1.51	13.11±1.55	12.17±1.38	9.89±1.51	10.49±1.47
	1.50	15.68±1.47	15.34±1.15	15.83±0.51	15.54±2.37	14.92±0.99	12.71±1.03	12.42±1.25
	2.00	19.67±0.81	18.03±1.12	18.91±0.82	18.42±1.60	18.50±0.98	16.01±0.42	15.71±0.49
6.5	2.50	25.56±0.70	23.83±0.87	25.11±1.70	24.27±1.56	24.45±1.59	21.72±0.94	21.09±1.10
	3.00	27.36±1.18	25.45±0.54	26.74±1.09	25.70±1.87	25.70±1.61	22.80±0.91	21.97±0.91
	3.50	29.43±1.01	27.70±1.14	28.45±0.45	27.20±1.77	28.13±0.98	24.67±1.73	23.44±1.25
	4.00	31.59±1.85	29.86±1.22	30.42±0.62	29.13±1.24	29.55±1.51	26.62±1.24	24.97±1.14
	4.50	33.48±2.52	31.75±1.01	32.66±0.88	31.15±0.90	31.03±0.99	28.47±1.40	26.94±1.18
	5.00	35.63±2.19	34.64±1.53	34.99±1.38	33.43±1.51	32.56±0.99	30.02±1.14	28.44±0.89
	5.50	37.85±1.91	36.35±1.55	36.86±1.19	35.44±1.45	34.89±1.14	31.81±1.24	30.36±1.22
	6.00	39.98±1.43	38.30±1.57	39.16±1.24	37.71±1.46	37.13±0.67	33.49±1.27	31.81±1.23
	6.50	43.48±1.44	40.87±2.20	41.36±1.55	39.69±1.17	39.27±1.09	35.03±1.52	33.48±1.27
	7.00	46.01±2.27	43.53±2.57	43.08±1.65	41.36±1.12	41.48±1.40	36.62±1.26	35.58±1.06
	7.50	48.80±2.04	45.74±2.42	45.78±2.02	43.82±1.46	43.02±1.15	38.81±1.00	37.38±1.36
	8.00	51.31±2.37	48.56±2.79	48.00±2.41	45.99±1.26	44.98±1.18	41.08±1.04	39.61±1.11
	8.50	54.28±2.14	51.47±2.67	50.11±2.50	48.23±1.10	46.98±1.22	42.86±1.17	41.36±1.19
	9.00	56.77±1.90	53.37±3.06	51.91±1.15	50.72±1.32	49.04±1.25	45.44±1.19	43.15±1.45
	9.50	59.34±1.65	56.23±2.74	54.48±1.72	53.10±1.33	51.35±1.59	47.53±0.78	44.81±1.25
	10.00	61.96±1.42	58.80±2.58	56.94±1.80	55.17±1.64	53.53±1.65	49.88±1.63	47.24±1.26
	10.50	64.47±1.42	61.25±2.64	59.09±1.63	57.47±1.62	55.95±1.50	52.28±1.97	49.38±1.30
	11.00	67.03±1.42	63.20±3.15	61.85±2.16	59.83±1.67	58.07±1.75	54.56±2.03	51.57±1.31
	11.50	69.65±1.12	66.66±2.88	64.68±2.22	62.43±1.86	60.42±1.81	57.28±1.78	53.82±1.35
	12.00	72.69±1.41	69.30±2.94	67.40±2.57	65.65±1.16	63.01±1.77	59.69±1.82	56.12±1.40

n=3*

TABLE 7B: COMPARISON OF *IN VITRO* RELEASE OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING POLYVINYL ALCOHOL 1% AS STABILIZER

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE						
		F8 EL 100	F9 EL 100	F10 EL 100	F11 EL 100	F12 EL 100	F13 EL 100	F14 EL 100
1.2	0.25	5.90±0.31	5.53±0.54	5.53±0.54	5.17±0.31	4.62±0.31	4.62±0.31	4.62±0.31
	0.50	8.38±0.84	8.00±0.57	8.00±1.12	7.43±1.09	6.31±0.56	6.31±0.54	6.31±0.56
	0.75	11.52±0.89	10.75±0.88	10.39±0.91	9.98±0.60	8.62±1.17	8.44±0.85	8.44±0.86
	1.00	15.15±1.23	14.17±0.92	13.24±0.62	12.81±0.38	11.03±1.47	10.84±0.58	10.84±0.98
	1.50	18.22±0.96	17.30±0.85	15.85±0.95	15.40±0.59	13.72±1.42	13.34±0.68	12.97±0.65
	2.00	21.40±0.70	20.33±0.78	18.92±0.69	18.46±1.13	17.25±1.34	16.66±0.79	15.73±0.68
6.5	2.50	28.36±1.21	27.27±1.20	25.30±1.12	24.90±1.08	22.60±1.92	22.14±1.11	21.15±0.91
	3.00	29.82±1.37	28.56±1.22	26.75±0.86	26.17±1.08	23.84±1.94	23.76±1.45	22.40±0.92
	3.50	32.27±1.19	30.08±1.22	28.45±1.13	27.51±1.08	25.69±1.96	25.26±1.65	24.07±1.10
	4.00	34.28±1.39	32.60±1.79	30.41±1.15	29.63±0.78	27.62±2.49	27.21±1.20	25.63±0.94
	4.50	36.55±1.61	35.23±1.83	32.45±1.16	31.67±0.77	29.27±2.33	28.49±1.11	27.26±1.27
	5.00	38.55±1.45	37.22±1.61	34.77±0.91	34.34±1.31	31.16±2.05	30.19±0.82	28.95±1.15
	5.50	40.25±1.47	39.29±1.85	36.62±0.70	36.01±1.33	33.32±2.06	32.14±1.12	30.89±1.31
	6.00	42.38±1.82	41.07±1.96	38.90±1.21	37.93±1.09	35.19±1.97	34.17±1.11	32.54±1.83
	6.50	44.95±2.02	43.27±1.79	40.90±2.09	40.09±1.22	37.12±2.29	36.27±1.40	34.24±1.38
	7.00	47.43±2.07	46.29±0.90	43.70±1.87	42.33±1.42	39.30±2.00	38.26±1.16	36.37±1.58
	7.50	49.81±1.96	48.86±1.22	45.86±1.64	44.64±1.67	41.37±2.27	39.94±0.78	38.38±1.43
	8.00	52.26±1.84	51.33±0.94	48.46±1.73	46.66±1.71	43.32±1.83	42.41±0.80	40.09±1.64
	8.50	55.34±1.71	53.87±0.96	50.96±1.89	49.10±1.98	45.70±1.97	44.59±0.63	42.59±1.67
	9.00	57.97±2.09	56.49±1.45	53.35±1.84	51.25±2.03	47.78±2.04	46.65±1.13	44.61±1.71
	9.50	60.85±2.09	59.00±0.97	55.80±2.20	53.63±2.37	50.10±1.99	49.33±1.17	46.88±1.56
	10.00	63.45±1.81	61.57±1.21	58.32±2.05	56.45±2.10	52.49±1.99	51.53±1.64	48.84±1.77
	10.50	66.12±1.52	64.20±1.28	61.08±1.97	58.99±1.88	55.31±2.00	53.97±1.54	51.03±1.81
	11.00	69.22±1.04	66.91±1.42	63.18±2.31	61.40±1.91	57.85±1.73	56.11±1.26	53.10±2.08
	11.50	72.03±0.71	69.67±1.62	65.87±1.81	64.06±1.69	60.45±1.77	58.47±1.47	55.20±2.21
	12.00	74.54±0.54	72.50±1.34	68.81±1.61	66.59±1.71	63.29±2.09	60.53±1.78	57.36±1.95

n=3*

TABLE 7C: COMPARISON OF *IN VITRO* RELEASE OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING PLURONIC F 68 1% AS STABILIZER

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE						
		F15 ES 100	F16 ES 100	F17 ES 100	F18 ES 100	F19 ES 100	F20 ES 100	F21 ES 100
1.2	0.25	5.90±0.31	5.72±0.31	5.53±0.54	5.53±0.54	5.72±0.31	4.81±0.31	4.62±0.31
	0.50	8.38±0.82	8.55±1.09	8.18±0.85	7.81±0.84	7.28±0.64	6.32±0.56	6.31±0.54
	0.75	11.33±0.84	11.15±1.14	10.40±0.35	9.83±0.85	9.63±1.30	8.45±0.67	8.62±0.64
	1.00	14.96±0.96	13.50±0.73	13.25±0.56	12.11±1.14	12.45±1.24	11.21±0.89	11.21±0.36
	1.50	18.20±0.62	16.66±0.65	15.49±1.13	15.21±0.82	14.84±1.24	13.36±0.93	13.54±0.58
	2.00	21.74±0.25	19.59±0.54	18.73±0.68	18.07±0.31	17.50±0.84	15.96±0.78	16.15±0.91
6.5	2.50	29.26±0.53	26.48±1.12	25.09±0.24	24.48±0.43	23.67±1.41	21.43±1.28	21.64±0.84
	3.00	31.12±1.03	28.32±1.14	26.72±0.41	25.76±0.46	25.30±1.59	22.68±1.29	22.89±0.42
	3.50	33.06±1.54	30.98±1.48	28.99±0.63	27.83±0.58	27.57±1.77	24.54±1.31	24.38±0.71
	4.00	35.46±1.45	32.84±1.44	30.80±1.00	30.18±1.09	29.57±1.59	26.29±1.19	25.93±1.02
	4.50	37.58±1.51	35.51±0.72	33.06±1.31	31.70±1.13	31.46±1.80	28.68±1.98	27.92±1.03
	5.00	39.80±1.18	37.37±0.64	35.22±1.13	33.65±1.33	33.62±1.85	30.43±1.70	29.43±1.21
	5.50	41.90±1.16	39.48±0.40	36.92±1.19	36.05±1.56	36.23±1.40	32.24±1.41	31.37±1.52
	6.00	43.90±0.69	42.03±0.62	39.40±1.57	38.17±1.45	38.01±1.29	33.93±1.93	33.39±1.74
	6.50	46.33±0.70	43.94±1.05	41.43±1.43	40.17±1.69	40.04±1.00	36.05±2.29	35.48±1.65
	7.00	49.04±1.40	46.09±1.26	43.52±1.82	42.43±1.57	41.57±1.18	37.87±1.94	37.09±1.60
	7.50	51.27±1.35	48.48±1.64	46.23±2.03	44.58±1.81	44.07±1.40	40.12±2.29	39.30±1.62
	8.00	54.13±1.35	50.95±2.02	48.85±2.44	46.97±1.69	46.46±1.46	41.88±2.12	41.40±1.66
	8.50	56.53±1.68	53.87±1.78	51.36±2.25	49.44±2.06	48.56±1.47	43.88±2.16	43.38±1.69
	9.00	59.36±1.19	56.69±2.28	53.95±2.40	51.80±2.41	50.53±1.73	46.30±2.52	45.23±1.47
	9.50	61.90±1.21	59.59±2.48	56.25±2.16	54.22±2.19	52.73±1.76	48.25±2.90	47.68±1.27
	10.00	64.51±1.51	62.21±2.27	58.78±2.53	56.71±2.34	54.99±1.78	50.23±2.34	49.83±1.28
	10.50	67.19±1.29	64.89±1.88	61.94±2.37	58.89±2.20	57.49±2.51	52.45±2.38	51.86±1.14
	11.00	69.92±1.63	67.45±1.93	64.44±1.90	61.12±2.16	60.24±2.42	54.73±2.42	53.93±1.46
	11.50	72.91±1.37	70.07±1.99	67.38±2.21	63.58±2.22	62.87±2.23	57.43±2.38	56.05±1.30
	12.00	75.60±1.40	73.12±1.96	70.38±2.33	66.29±1.96	65.20±1.97	60.02±2.67	58.21±1.18

n=3*

TABLE 7D: COMPARISON OF *IN VITRO* RELEASE OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING POLYVINYL ALCOHOL 1% AS STABILIZER

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE						
		F22 ES 100	F23 ES 100	F24 ES 100	F25 ES 100	F26 ES 100	F27 ES 100	F28 ES 100
1.2	0.25	5.90±0.31	5.90±0.31	5.35±0.31	5.35±0.31	5.35±0.31	4.81±0.31	4.62±0.31
	0.50	8.56±1.09	8.74±0.81	7.44±0.56	7.44±0.53	7.44±0.54	6.87±0.54	6.13±0.32
	0.75	11.52±0.85	11.35±0.86	10.71±0.85	10.53±0.54	10.35±0.66	9.20±1.16	7.88±0.33
	1.00	14.98±1.47	14.98±1.06	13.40±0.70	13.40±0.82	12.66±0.60	11.09±0.92	10.07±0.32
	1.50	17.85±1.21	18.40±0.50	16.93±0.67	16.01±1.13	15.24±0.35	13.05±0.67	12.17±0.87
	2.00	21.56±0.94	21.22±0.27	20.06±0.57	18.91±0.64	18.10±0.37	16.00±0.86	15.26±0.69
6.5	2.50	28.51±1.12	28.74±0.57	26.72±0.81	25.28±0.50	24.35±0.73	21.50±0.72	20.36±1.02
	3.00	30.35±1.32	30.23±0.77	28.37±1.46	26.92±0.75	25.80±1.05	23.12±1.20	21.78±1.12
	3.50	33.37±1.82	32.71±0.80	30.46±1.84	28.81±1.64	27.87±0.88	25.37±1.36	23.46±0.98
	4.00	35.05±2.02	34.56±0.81	32.45±1.90	30.79±1.69	29.67±0.58	27.16±1.77	25.21±1.31
	4.50	37.17±1.59	36.85±1.39	35.09±1.93	33.04±2.01	31.53±1.23	28.84±2.07	27.03±1.16
	5.00	39.56±1.81	39.05±1.84	37.28±2.46	34.64±2.19	33.85±1.15	30.96±1.58	28.55±1.18
	5.50	41.85±1.48	41.15±1.82	39.19±2.40	36.48±2.04	35.88±1.44	32.79±1.89	30.32±0.99
	6.00	43.86±1.54	43.33±2.20	41.16±1.84	38.57±2.21	38.18±1.50	34.69±1.43	32.33±1.29
	6.50	46.49±1.67	45.57±2.23	43.75±2.20	40.92±1.86	40.37±2.14	36.65±1.55	33.87±1.61
	7.00	48.84±2.12	48.08±2.14	46.24±1.87	42.79±1.91	42.63±1.93	38.67±1.79	35.63±1.65
	7.50	51.44±1.62	50.49±1.81	48.46±1.67	45.28±2.36	44.79±1.75	40.76±1.93	38.20±1.95
	8.00	53.58±1.59	53.15±1.68	51.10±1.69	47.67±2.12	47.01±1.20	42.92±1.65	40.30±2.06
	8.50	55.77±1.61	56.08±1.65	53.64±1.96	50.31±2.17	49.48±1.83	45.14±1.55	42.09±1.82
	9.00	58.76±1.84	58.55±1.47	56.08±1.76	52.49±1.85	51.47±1.58	47.42±1.61	44.30±1.82
	9.50	60.92±1.66	60.90±1.51	58.58±2.10	54.90±1.64	53.69±1.62	49.77±1.34	46.58±1.61
	10.00	63.67±2.14	63.49±1.46	61.14±2.07	57.38±1.43	56.33±1.73	52.00±1.35	48.92±1.42
	10.50	66.32±1.90	65.96±1.49	63.58±2.11	59.74±1.64	58.68±1.77	54.29±1.72	51.32±1.77
	11.00	69.40±1.72	68.49±1.92	66.26±2.45	61.97±1.41	61.27±1.74	56.63±1.75	53.79±1.70
	11.50	72.38±1.46	71.07±2.12	68.64±2.21	64.80±1.20	63.93±1.54	59.21±1.47	56.14±1.74
	12.00	75.62±1.48	73.52±1.89	71.80±1.70	67.52±1.43	66.46±1.57	61.85±1.59	58.55±1.78

n=3*

TABLE 7E: COMPARISON OF *IN VITRO* DRUG RELEASE OF PURE DRUG (FELODIPINE) WITH BEST FORMULATION (F7 EL 100 with 0.1% SLS)

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE	
		PURE DRUG	F7 EL 100 + 1% PLURONIC F 68
1.2	0.25	0	0
	0.5	0	0
	0.75	0	0
	1.00	0	0
	1.50	0	0
	2.00	0	0
6.5	2.50	1.42±0.32	11.42±0.64
	3.00	2.60±0.31	14.22±0.28
	3.50	4.40±0.32	17.12±0.86
	4.00	5.72±0.32	20.32±0.36
	4.50	6.54±0.33	22.72±0.25
	5.00	7.58±0.67	25.19±0.05
	5.50	8.83±0.38	27.36±0.53
	6.00	9.76±0.40	29.96±0.81
	6.50	-	32.08±1.17
	7.00	-	34.06±1.35
	7.50	-	36.26±1.29
	8.00	-	38.32±1.01
	8.50	-	40.61±0.87
	9.00	-	42.38±0.81
	9.50	-	44.73±0.61
	10.00	-	46.94±0.30
	10.50	-	49.00±0.37
	11.00	-	51.28±0.08
	11.50	-	53.22±0.27
	12.00	-	55.37±0.55

n=3*

TABLE 8A: KINETICS RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING PLURONIC F 68 1% AS STABILIZER

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORS-MEYER & PEPPAS MODEL		HIXON – CROWELL MODEL	
	R^2	K_0 h^{-1}	R^2	K_1 h^{-1}	R^2	K_H $h^{1/2}$	R^2	n	R^2	K_{HC} $h^{1/3}$
F1	0.988	5.582	0.981	-0.042	0.981	22.77	0.994	0.701	0.991	-0.122
F2	0.990	5.275	0.983	-0.038	0.980	21.46	0.996	0.676	0.993	-0.111
F3	0.982	5.074	0.987	-0.035	0.987	20.72	0.995	0.680	0.992	-0.107
F4	0.984	4.889	0.986	-0.033	0.984	19.90	0.994	0.663	0.992	-0.101
F5	0.979	4.713	0.989	-0.031	0.988	19.23	0.995	0.661	0.992	-0.096
F6	0.985	4.475	0.986	-0.029	0.980	18.23	0.991	0.673	0.990	-0.089
F7	0.984	4.182	0.990	-0.026	0.982	17.00	0.995	0.647	0.993	-0.081

TABLE 8B: KINETICS RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING POLYVINYL ALCOHOL 1% AS STABILIZER

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORS-MEYER & PEPPAS MODEL		HIXON – CROWELL MODEL	
	R^2	K_0 h^{-1}	R^2	K_1 h^{-1}	R^2	K_H $h^{1/2}$	R^2	n	R^2	K_{HC} $h^{1/3}$
F8	0.982	5.558	0.975	-0.043	0.982	22.59	0.995	0.646	0.987	-0.124
F9	0.984	5.439	0.980	-0.041	0.983	22.13	0.996	0.658	0.990	-0.119
F10	0.986	5.157	0.983	-0.037	0.981	20.95	0.995	0.649	0.991	-0.110
F11	0.985	4.988	0.984	-0.035	0.982	20.29	0.996	0.656	0.991	-0.104
F12	0.986	4.738	0.984	-0.031	0.980	19.29	0.995	0.679	0.990	-0.096
F13	0.986	4.588	0.987	-0.030	0.981	18.68	0.995	0.673	0.992	-0.092
F14	0.986	4.327	0.990	-0.027	0.981	17.59	0.995	0.657	0.993	-0.085

TABLE 8C: KINETICS RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING PLURONIC F 68 1% AS STABILIZER

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORS-MEYER & PEPPAS MODEL		HIXON – CROWELL MODEL	
	R^2	K_0 h^{-1}	R^2	K_1 h^{-1}	R^2	K_H $h^{1/2}$	R^2	n	R^2	K_{HC} $h^{1/3}$
F15	0.980	5.659	0.978	-0.044	0.987	23.06	0.996	0.655	0.989	-0.127
F16	0.985	5.493	0.979	-0.041	0.982	22.34	0.996	0.655	0.990	-0.121
F17	0.987	5.258	0.978	-0.038	0.979	21.35	0.995	0.653	0.989	-0.113
F18	0.986	4.999	0.988	-0.035	0.983	20.33	0.995	0.650	0.993	-0.104
F19	0.985	4.903	0.988	-0.033	0.985	19.95	0.994	0.649	0.994	-0.102
F20	0.987	4.504	0.990	-0.029	0.983	18.33	0.995	0.664	0.994	-0.090
F21	0.985	4.397	0.991	-0.028	0.983	17.89	0.996	0.658	0.994	-0.087

TABLE 8D: KINETICS RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING POLYVINYL ALCOHOL 1% AS STABILIZER

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORS-MEYER & PEPPAS MODEL		HIXON – CROWELL MODEL	
	R^2	K_0 h^{-1}	R^2	K_1 h^{-1}	R^2	K_H $h^{1/2}$	R^2	n	R^2	K_{HC} $h^{1/3}$
F22	0.980	5.612	0.978	-0.044	0.987	22.86	0.997	0.651	0.989	-0.126
F23	0.980	5.528	0.984	-0.042	0.988	22.51	0.996	0.645	0.992	-0.123
F24	0.983	5.408	0.984	-0.040	0.986	22.05	0.996	0.669	0.992	-0.118
F25	0.983	5.039	0.985	-0.035	0.984	20.49	0.996	0.650	0.991	-0.106
F26	0.986	4.993	0.986	-0.035	0.983	20.30	0.996	0.652	0.993	-0.104
F27	0.988	4.658	0.988	-0.030	0.980	18.94	0.995	0.664	0.993	-0.094
F28	0.989	4.411	0.986	-0.028	0.976	17.93	0.993	0.672	0.991	-0.087

TABLE 9: COMPARISON OF SOLUBILITY OF BEST FORMULATION (F7 EL 100 with 1% PLURONIC F68) WITH PURE DRUG USING DISTILLED WATER WITH 0.1% SLS AND PHOSPHATE BUFFER pH 6.5 WITH 0.1% SLS

TIME (hrs)	SOLVENT USED	SOLUBILITY (µg/ml)	
		PURE DRUG	F7 EL 100 + PLURONIC F 68
24 hrs	Distilled water	29.91µg/ml±3.218	117.23µg/ml±3.215
	Phosphate buffer pH 6.5 with 0.1% SLS	43.89µg/ml±3.207	260.58µg/ml±3.210

n=3*

TABLE 10A: COMPARISON OF CUMULATIVE AMOUNT OF DRUG PERMEATED ACROSS DUODENUM SEGMENT

TIME(HRS)	CUMULATIVE AMOUNT OF DRUG PERMEATED(mg)± SD	
	PURE DRUG SOLUTION	F7 EL 100 + 1% PLURONIC F 68
0.25	0.1036±0.008	0.2105±0.013
0.50	0.1373±0.008	0.3655±0.042
1.00	0.1708±0.016	0.5617±0.045
1.50	0.1991±0.016	0.7298±0.021
2.00	0.2219±0.011	0.8572±0.011

n=3*

TABLE 10B: COMPARISON OF CUMULATIVE AMOUNT OF DRUG PERMEATED ACROSS JEJUNUM SEGMENT

TIME(HRS)	CUMULATIVE AMOUNT OF DRUG PERMEATED(mg)± SD	
	PURE DRUG SOLUTION	F7 EL 100 + 1% PLURONIC F 68
0.25	0.1125±0.010	0.2266±0.026
0.50	0.1484±0.013	0.4287±0.038
1.00	0.1874±0.019	0.5916±0.041
1.50	0.2231±0.011	0.7882±0.055
2.00	0.2533±0.008	0.8975±0.016

n=3*

TABLE 10C: COMPARISON OF CUMULATIVE AMOUNT OF DRUG PERMEATED ACROSS ILEUM SEGMENT

TIME(HRS)	CUMULATIVE AMOUNT OF DRUG PERMEATED(mg)± SD	
	PURE DRUG SOLUTION	F7 EL 100 + 1% PLURONIC F 68
0.25	0.1071±0.010	0.2194±0.016
0.50	0.1446±0.014	0.4158±0.050
1.00	0.1765±0.013	0.5731±0.059
1.50	0.2065±0.011	0.7517±0.032
2.00	0.2329±0.011	0.8743±0.013

n=3*

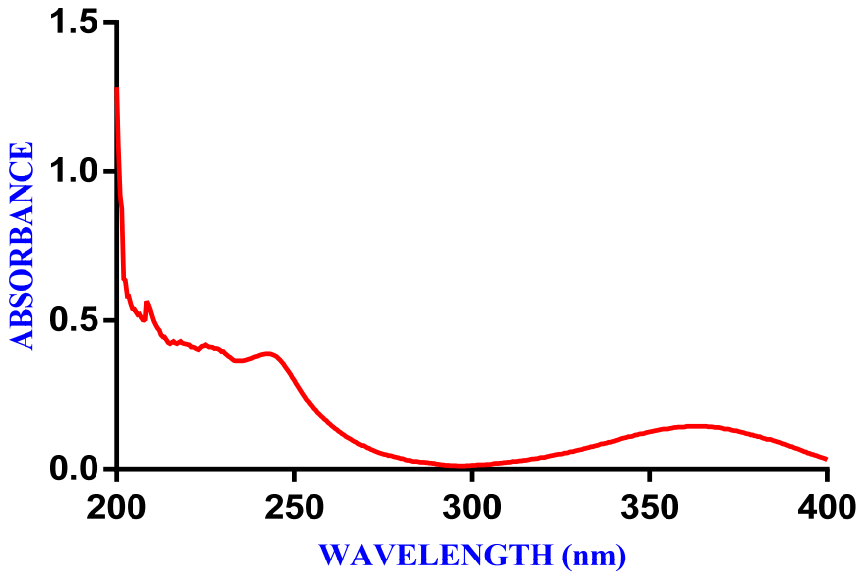

FIGURE 20a: λMAX OF FELODIPINE USING DISTILLED WATER WITH 0.1% SLS

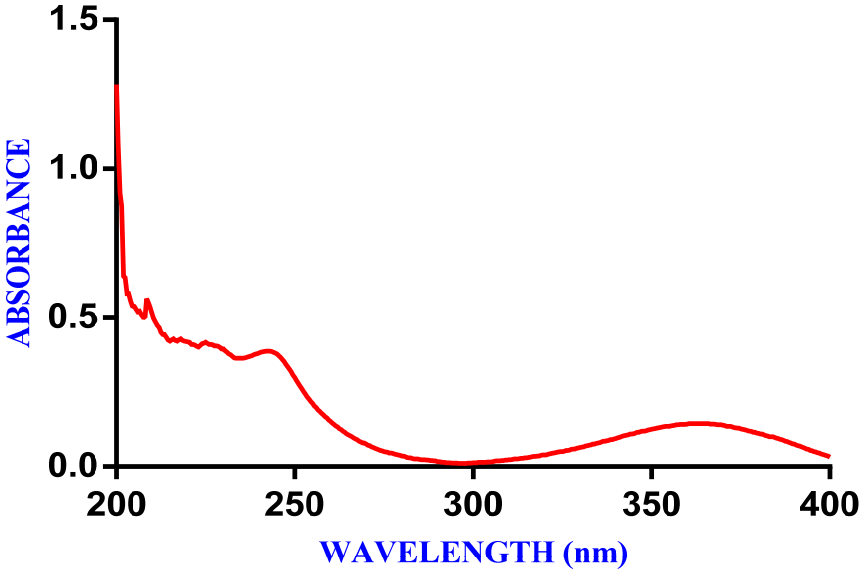

FIGURE 20b: λMAX OF FELODIPINE USING 0.1N HYDROCHLORIC ACID WITH 0.1% SLS

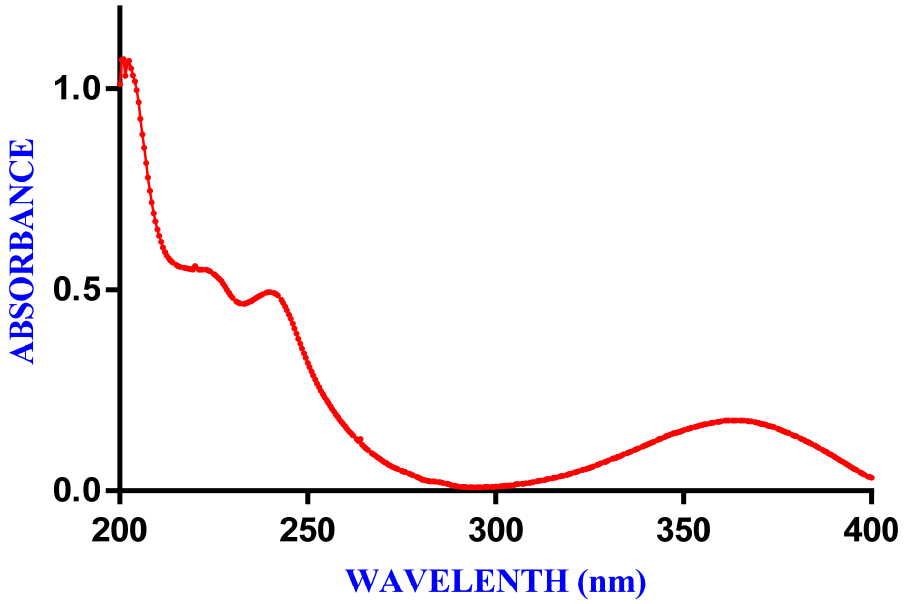

FIGURE 20c: λMAX OF FELODIPINE USING PHOSPHATE BUFFER PH 6.5 WITH 0.1% SLS

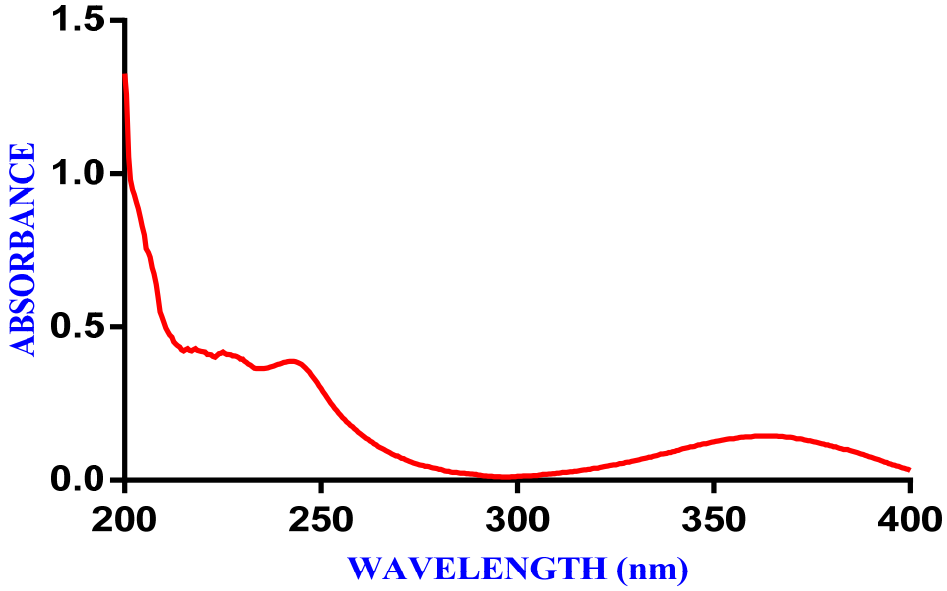

FIGURE 20d: λMAX OF FELODIPINE USING PHOSPHATE BUFFER PH 6.5 WITH 0.1% SLS

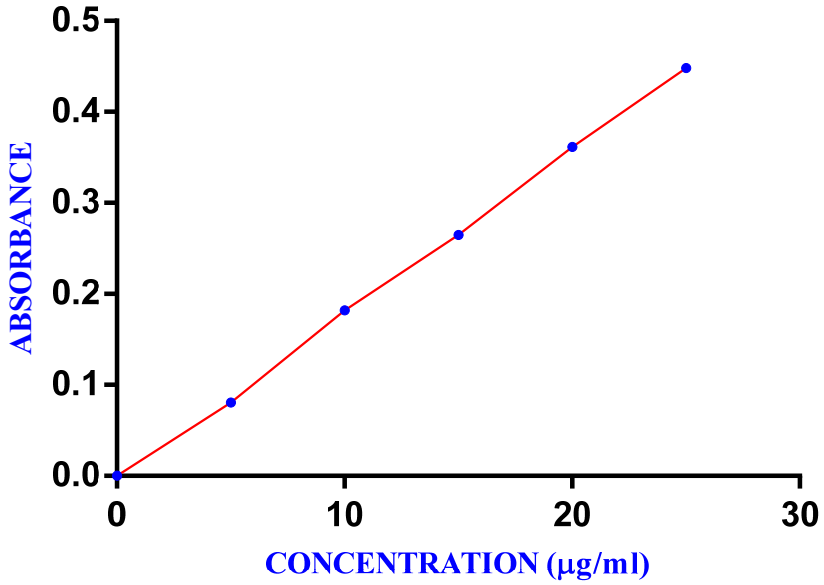

FIGURE 20e: CALIBRATION OF FELODIPINE USING DISTILLED WATER WITH 0.1% SLS

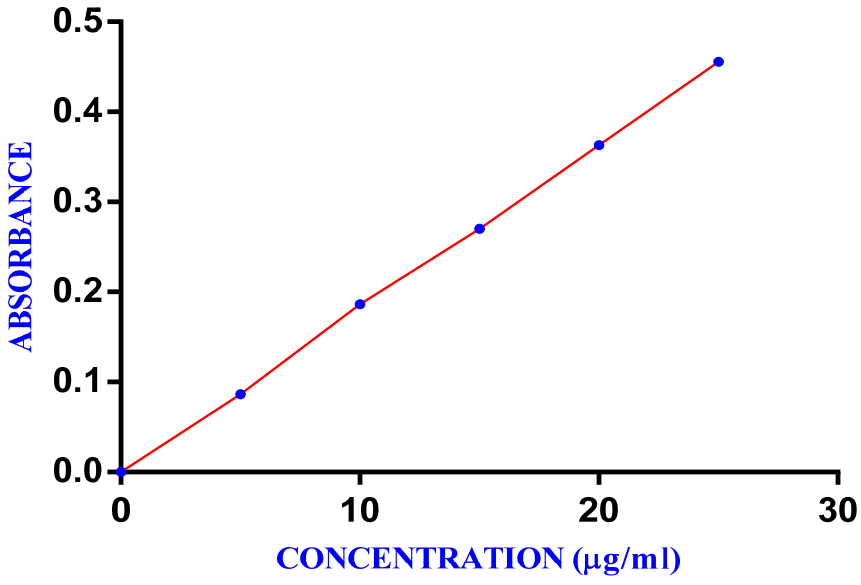

FIGURE 20f: CALIBRATION OF FELODIPINE USING 0.1N HYDROCHLORIC ACID WITH 0.1% SLS

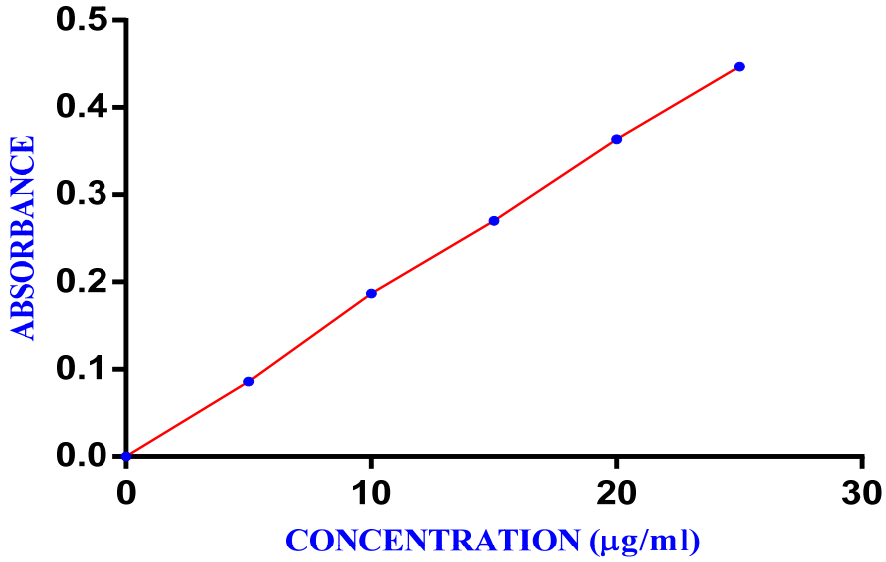

FIGURE 20g: CALIBRATION OF FELODIPINE USING PHOSPHATE BUFFER PH 6.5 WITH 0.1% SLS

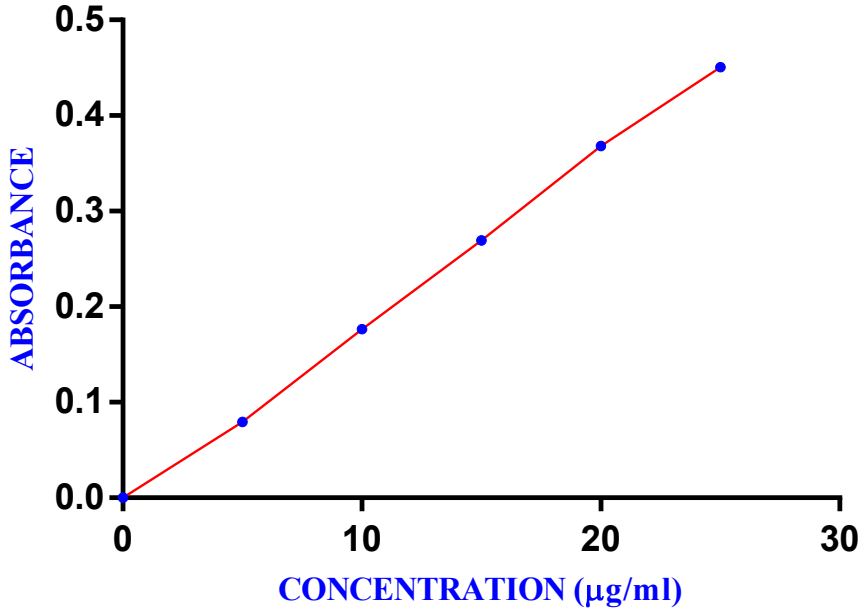

FIGURE 20h: CALIBRATION OF FELODIPINE USING PHOSPHATE BUFFER PH 7.4 WITH 0.1% SLS

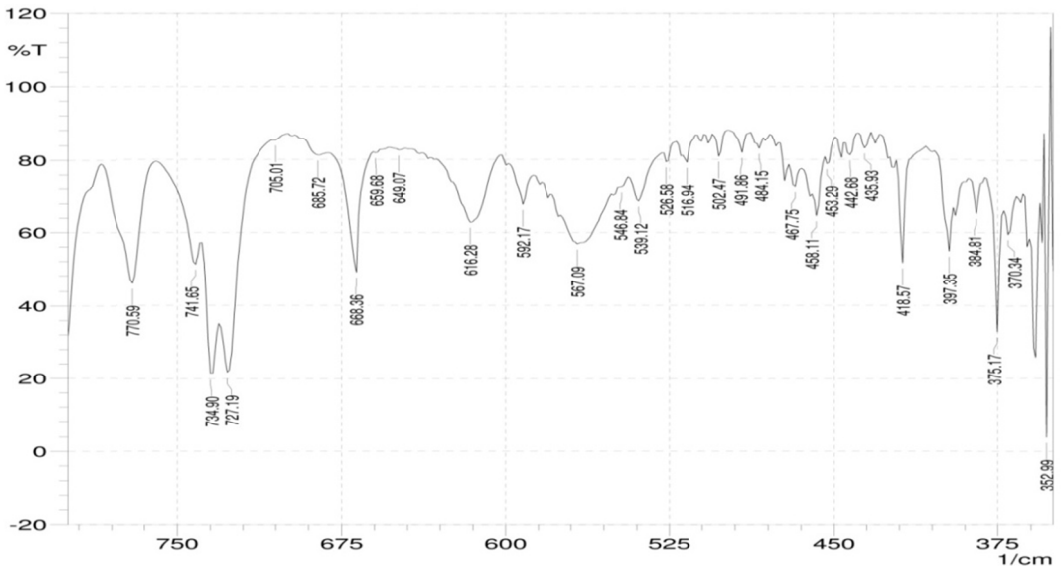

FIGURE 21a: INFRARED SPECTRAM OF FELODIPINE

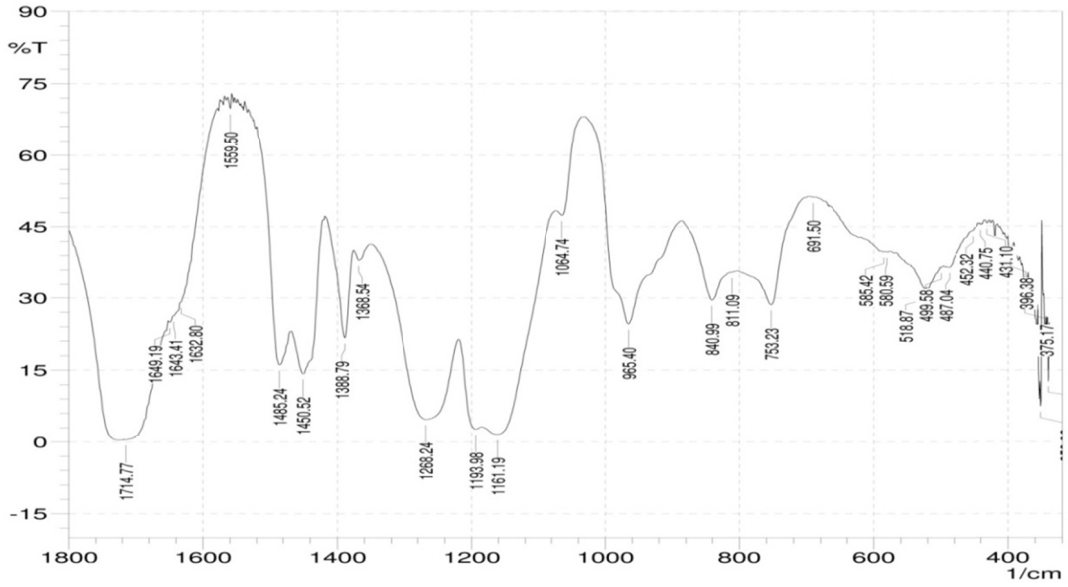

FIGURE 21b: INFRARED SPCTRUM OF EUDRAGIT L 100

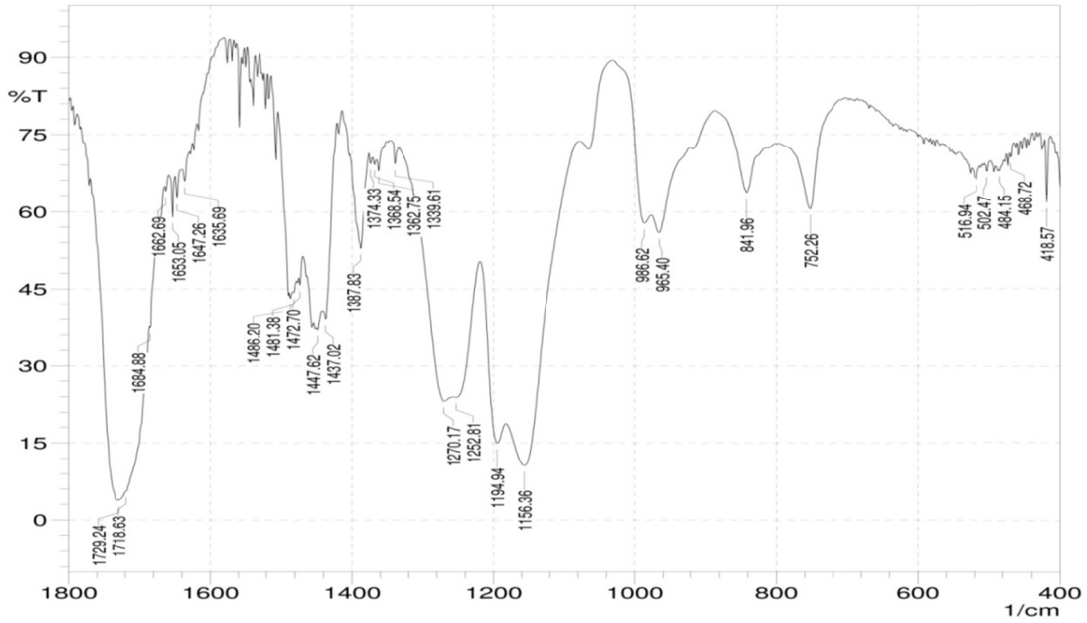

FIGURE 21c: INFRARED SPECTRAM OF EUDRAGIT S 100

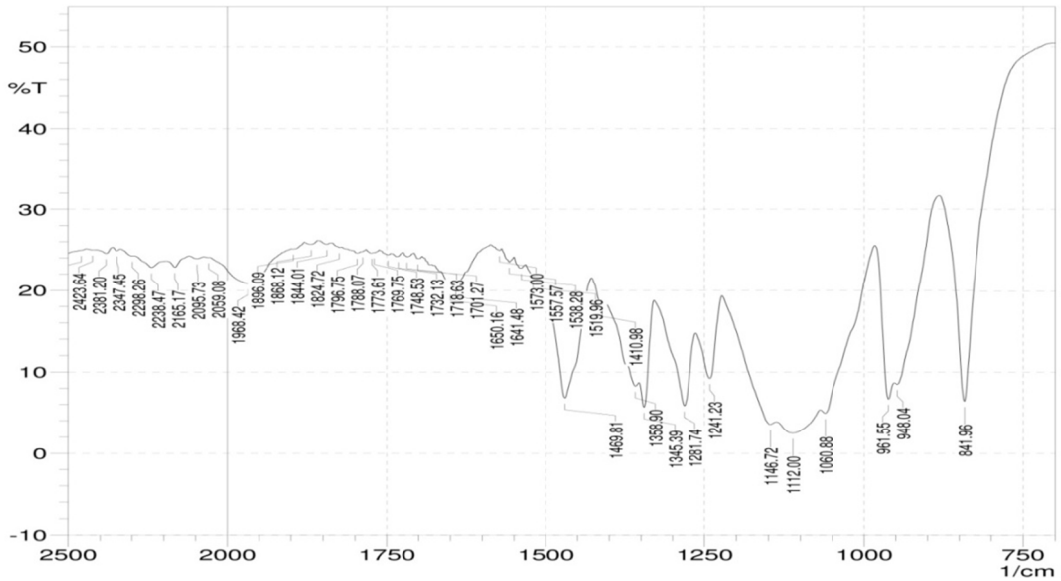

FIGURE 21d: INFRARED SPECTRAM OF PLURONIC F 68

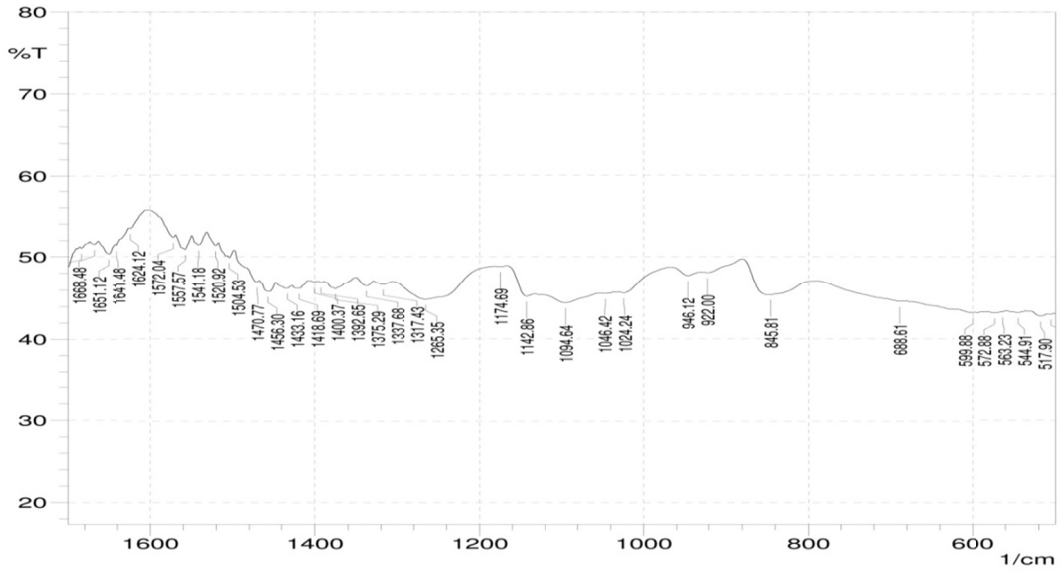

FIGURE 21e: INFRARED SPECTAM OF POLYVINYL ALCOHOL

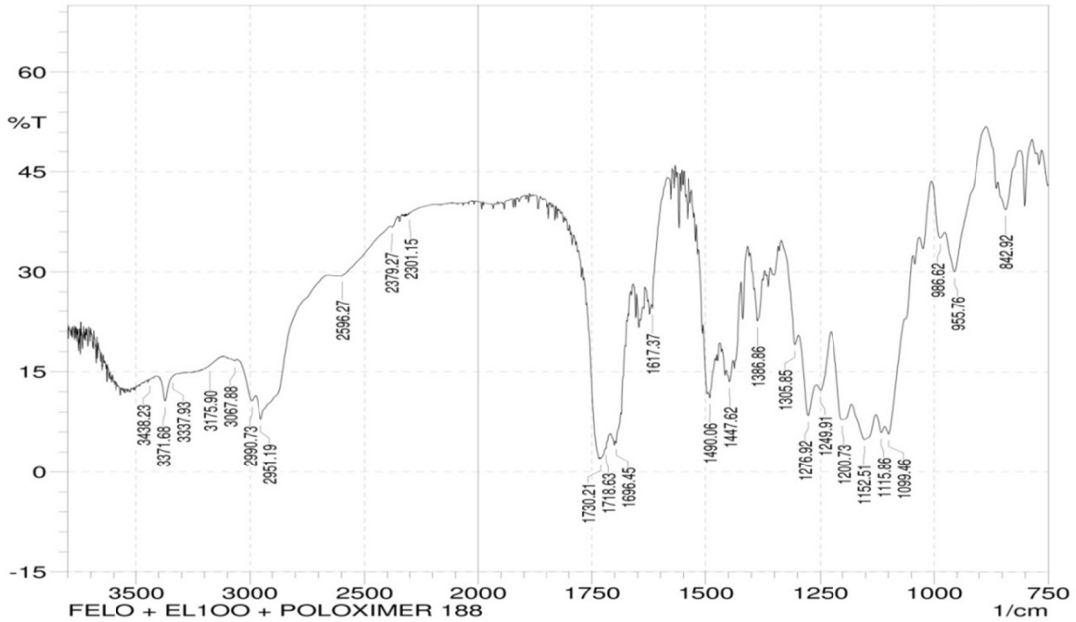

FIGURE 21f: INFRARED SPECTAM OF FELODIPINE + EUDRAGIT L 100+ POLOXAMER 188

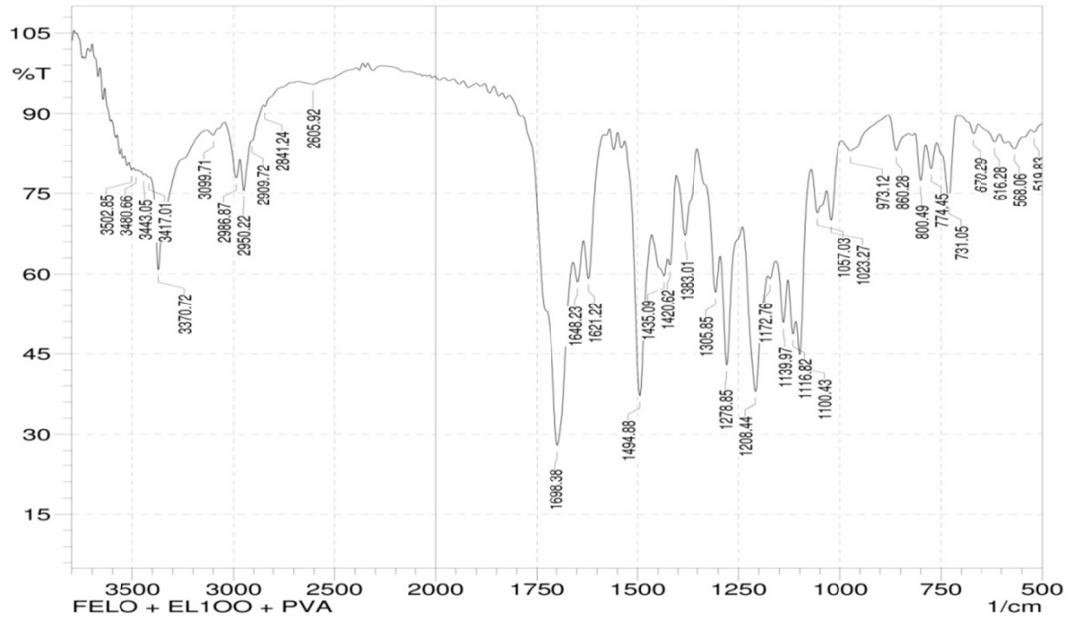

FIGURE 21g: INFRARED SPECTAM OF FELODIPINE + EUDRAGIT L 100+ POLYVINYL ALCOHOL

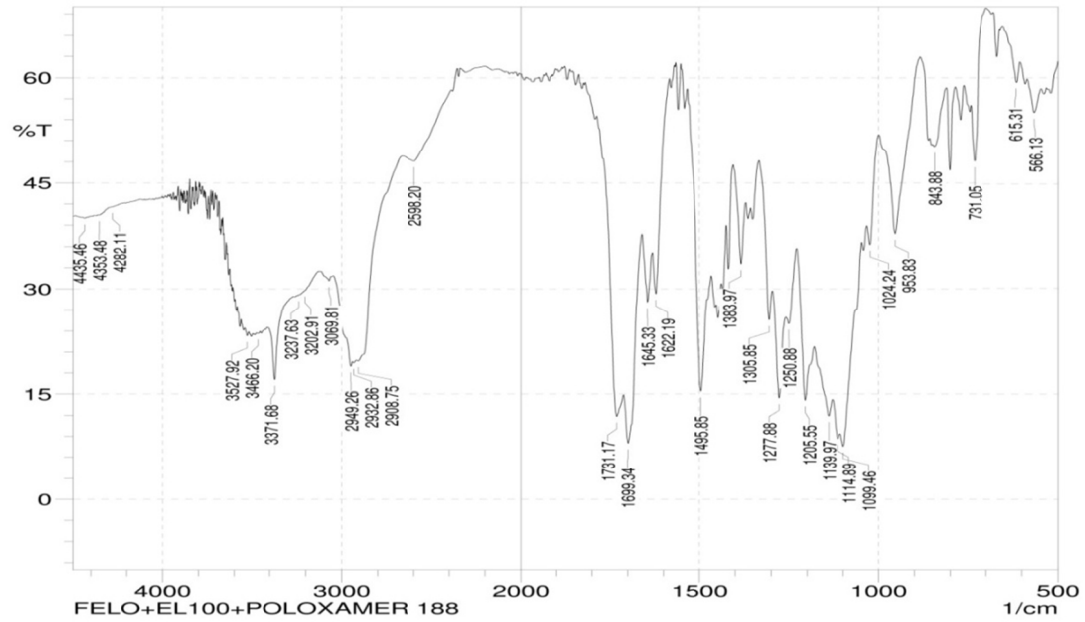

FIGURE 21h: INFRARED SPECTAM OF FELODIPINE + EUDRAGIT S 100+ POLOXAMER 188

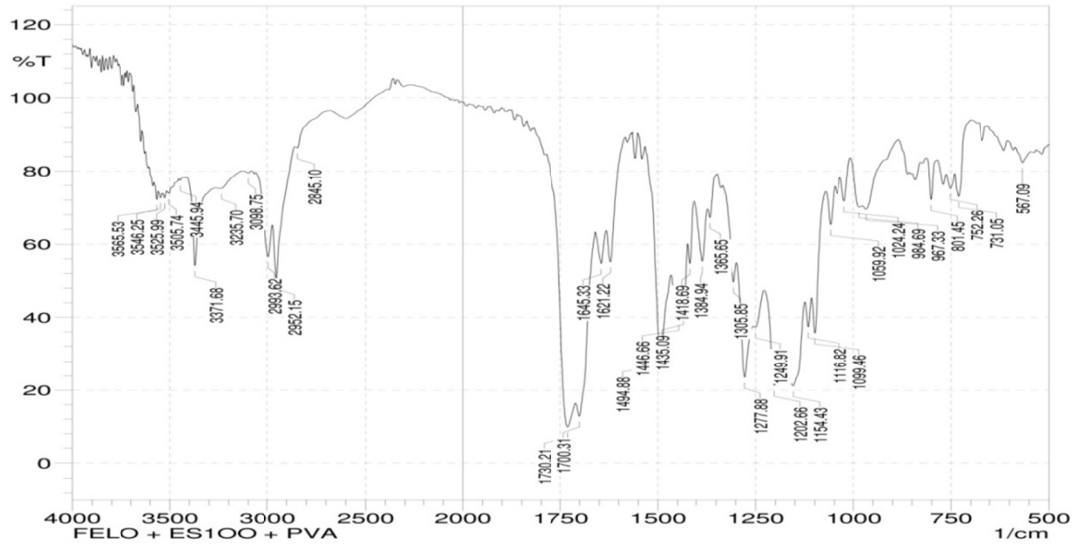

FIGURE 21i: INFRARED SPECTAM OF FELODIPINE + EUDRAGIT S 100+ POLYVINYL ALCOHOL

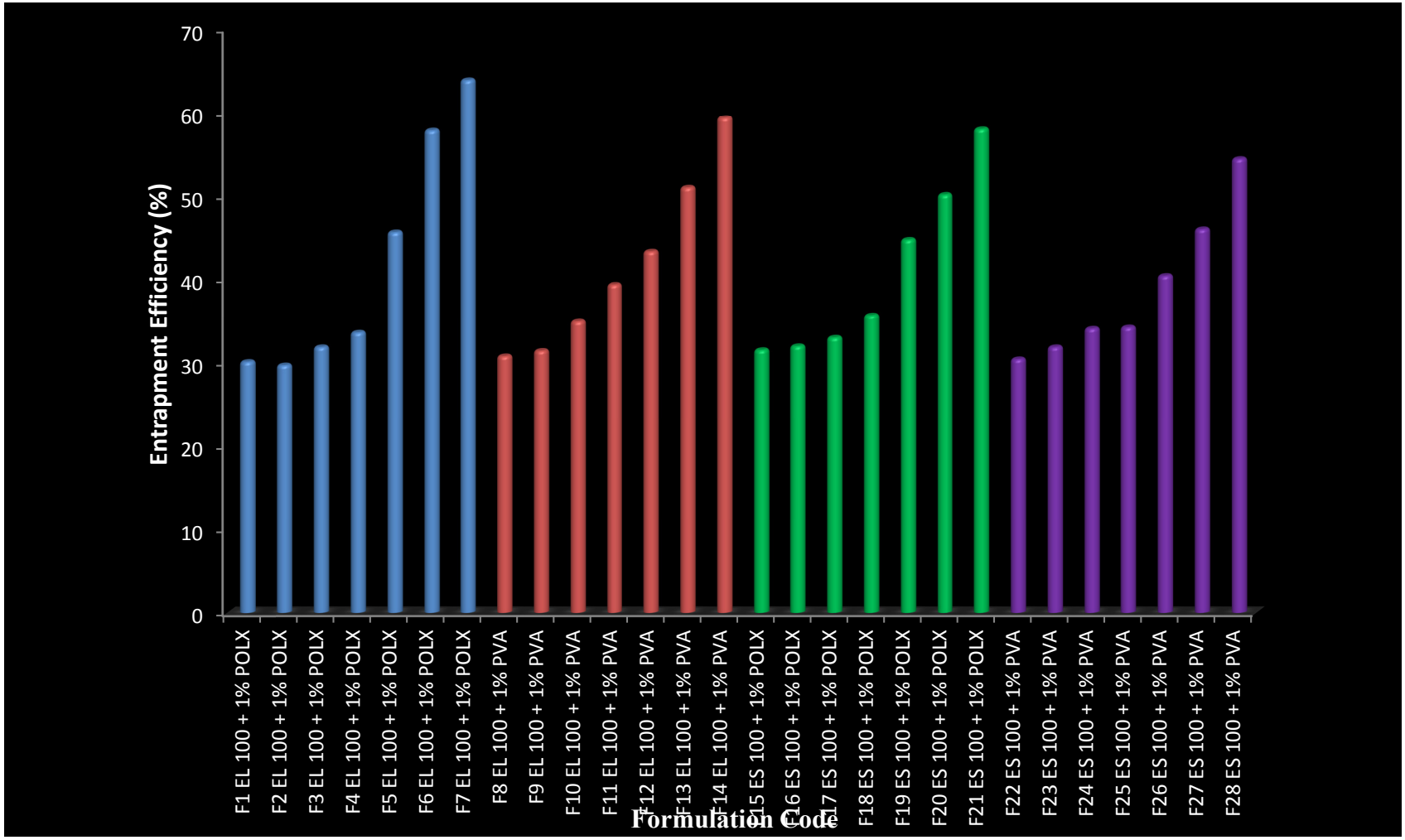

FIGURE 22: PERCENTAGE ENTRAPMENT EFFICIENCY OF FELODIPINE POLYMERIC NANOPARTICLES

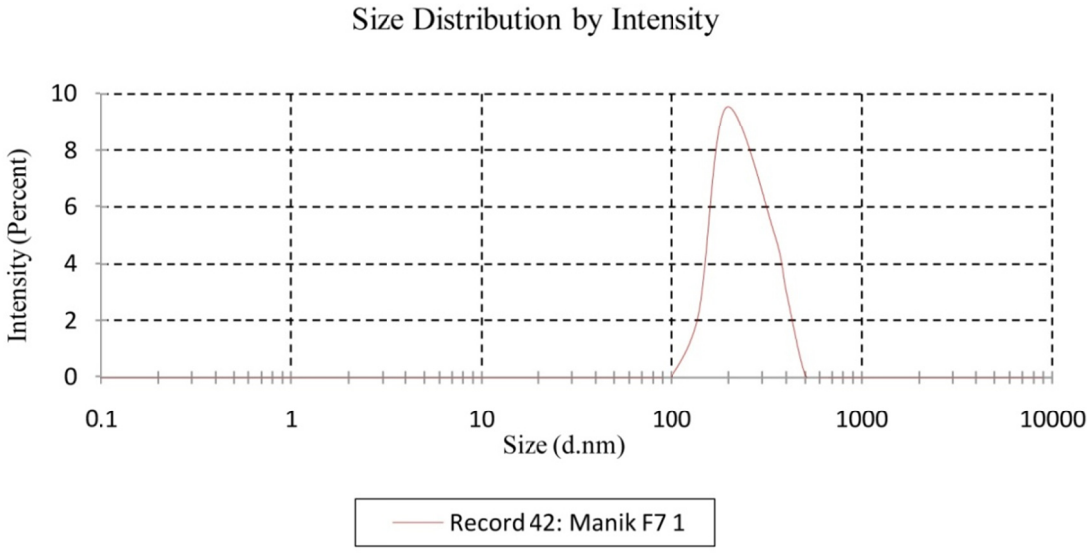

FIGURE 23 a: PARTICLE SIZE DISTRIBUTION CURVE OF F7 EL 100 + 1% PLURONIC F 68

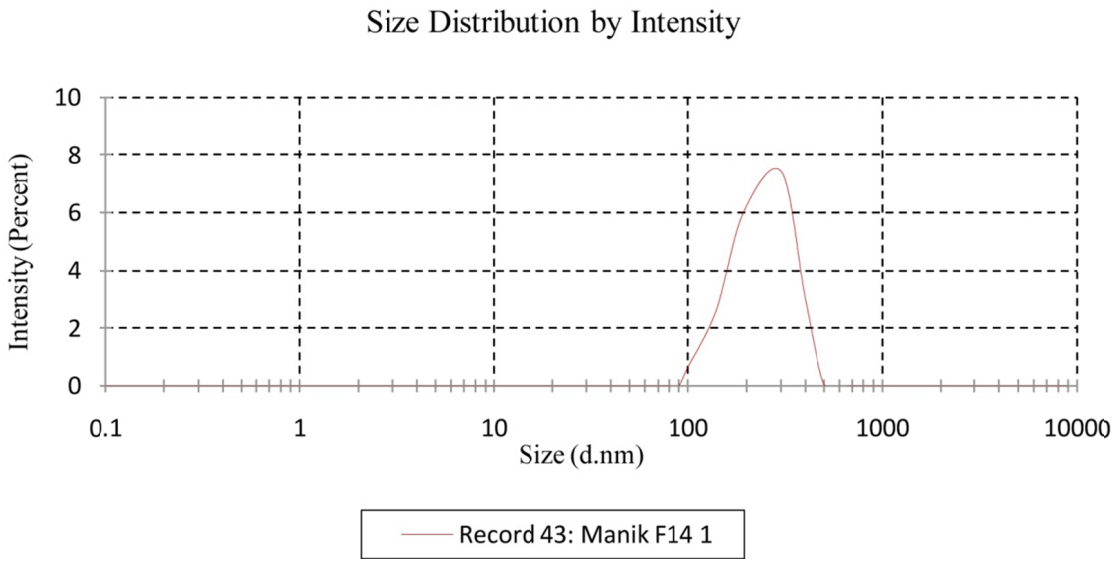

FIGURE 23b: PARTICLE SIZE DISTRIBUTION CURVE OF F14 EL 100 + 1% POLYVINYL ALCOHOL

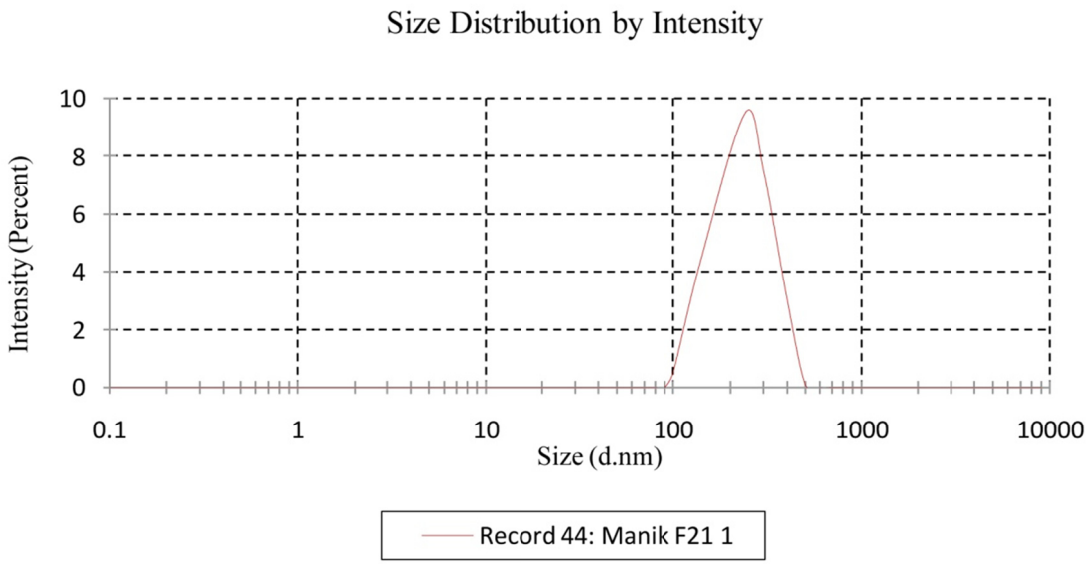

FIGURE 23c: PARTICLE SIZE DISTRIBUTION CURVE OF F21 ES 100 + 1% PLURONIC F 68

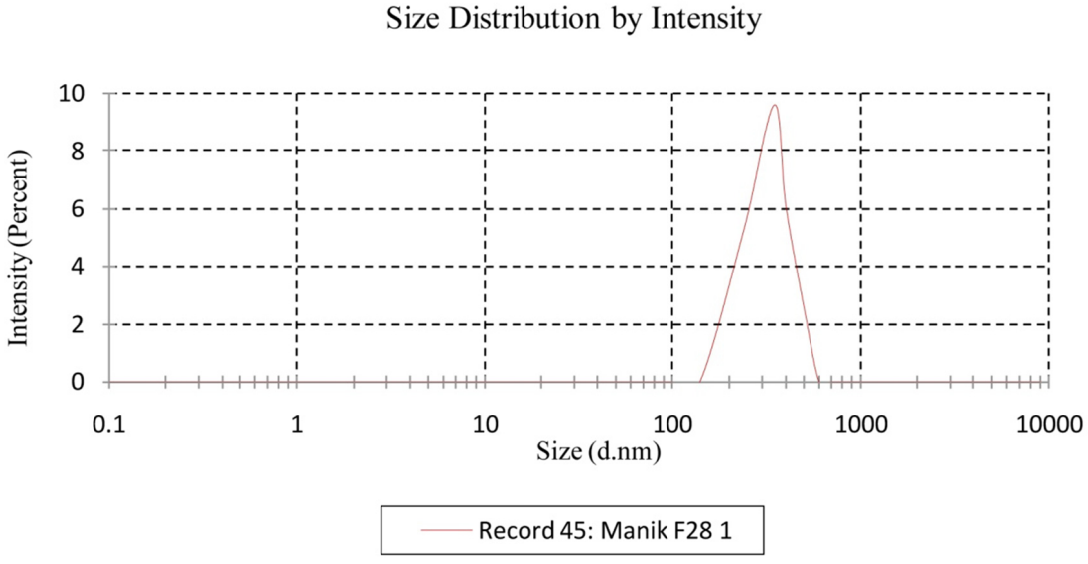

FIGURE 23d: PARTICLE SIZE DISTRIBUTION CURVE OF F28 ES 100 + 1% POLYVINYL ALCOHOL

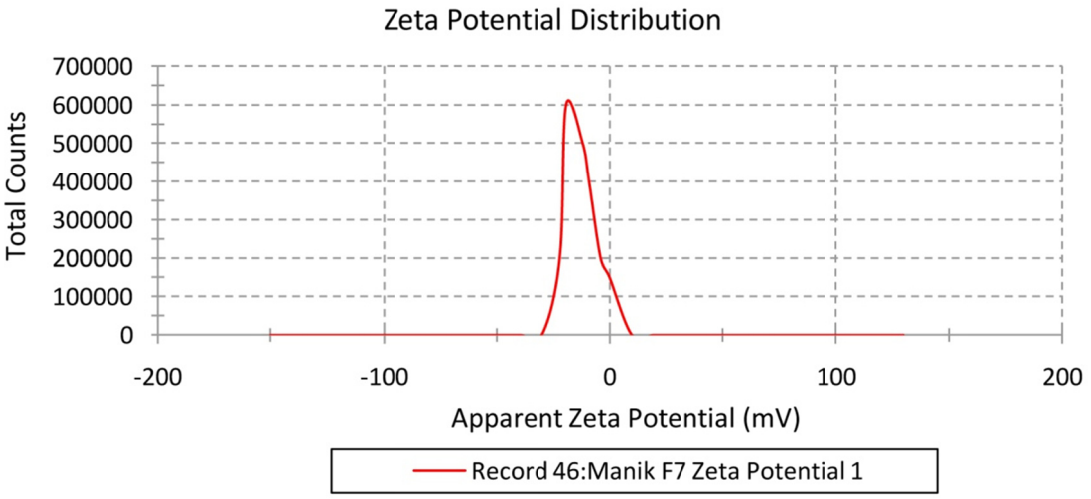

FIGURE 24a: ZETA POTENTIAL OF FORMULATION F7 EL 100 + 1% PLURONIC F 68

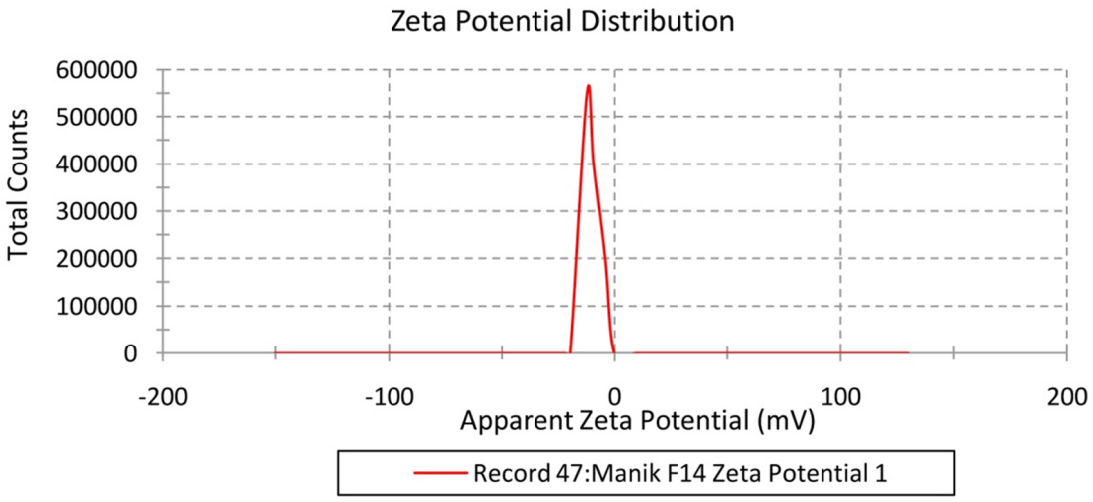

FIGURE 24b: ZETA POTENTIAL OF FORMULATION F14 EL 100 + 1% POLYVINYL ALCOHOL

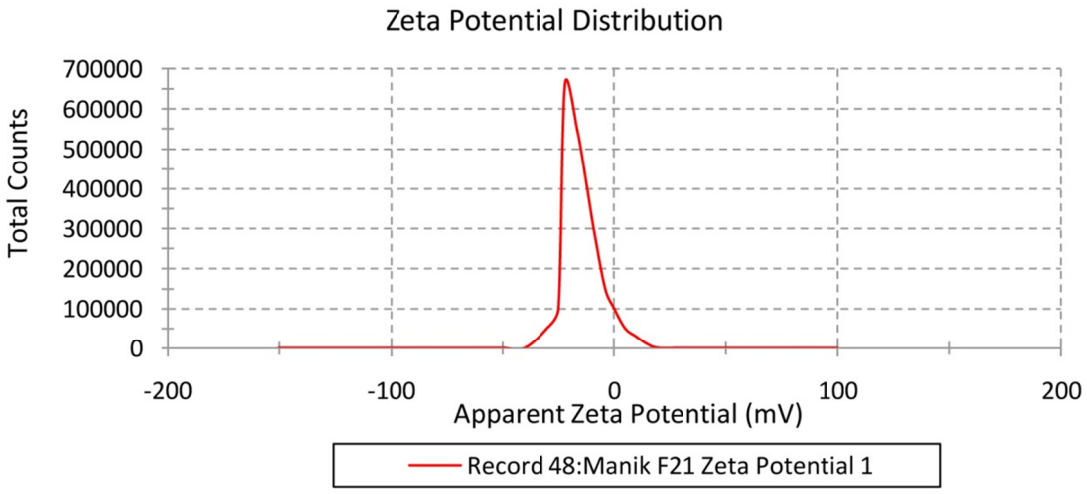

FIGURE 24c: ZETA POTENTIAL OF FORMULATION F21 ES 100 + 1% PLURONIC F 68

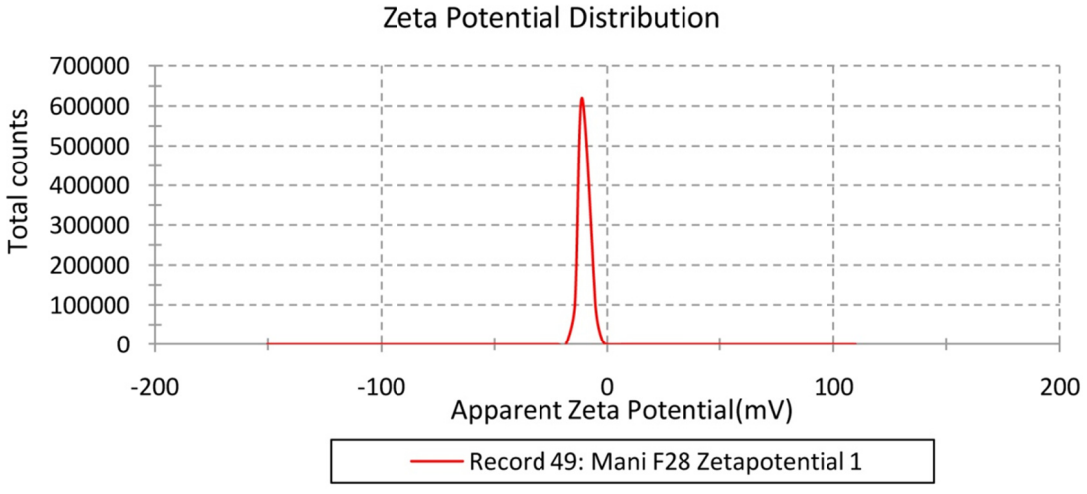

FIGURE 24d: ZETA POTENTIAL OF FORMULATION F28 EL 100 + 1% POLYVINYL ALCOHOL

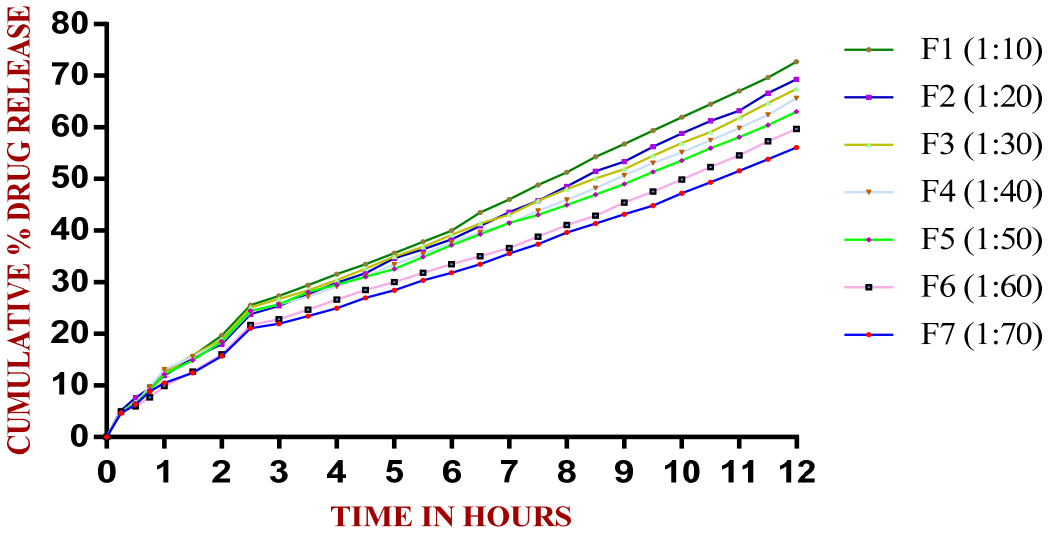

FIGURE 25 a: INVITRO RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

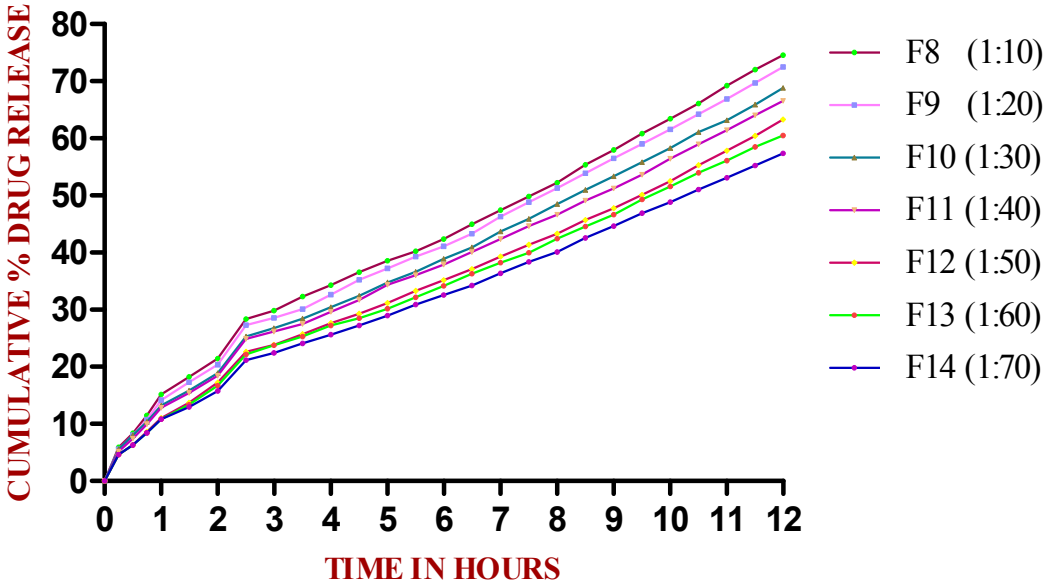

FIGURE 25b: INVITRO RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT L100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

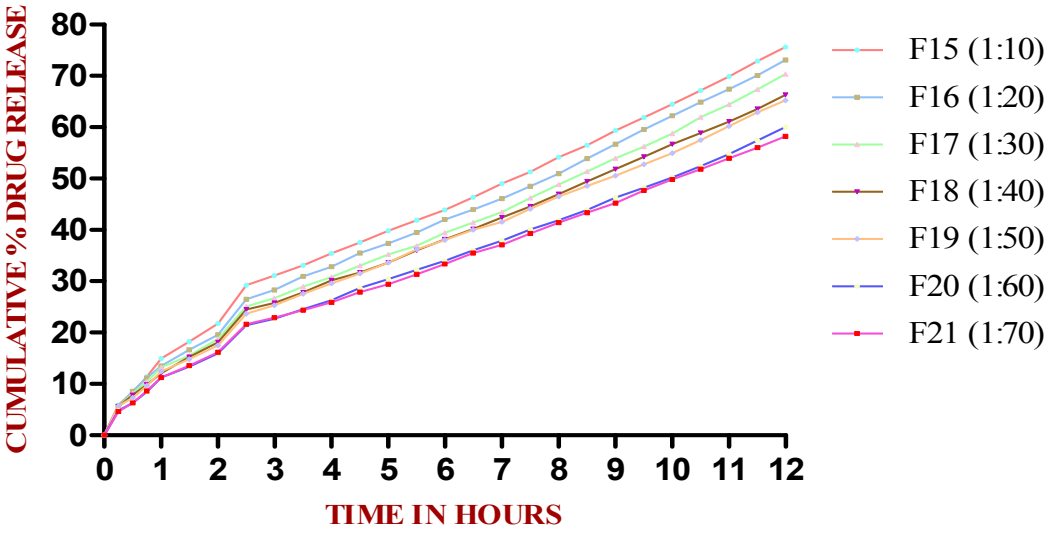

FIGURE 25c: INVITRO RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

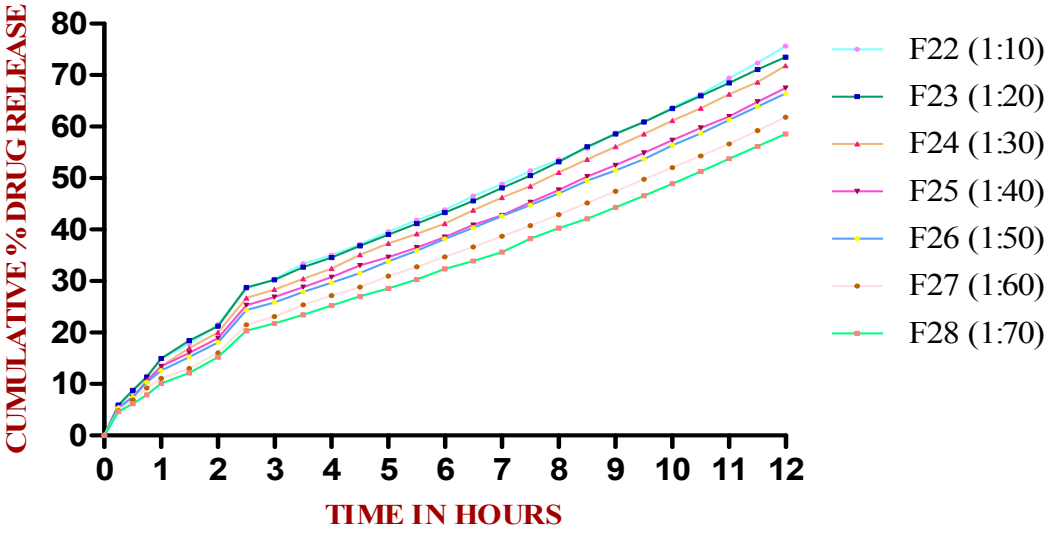

FIGURE 25d: INVITRO RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT S100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

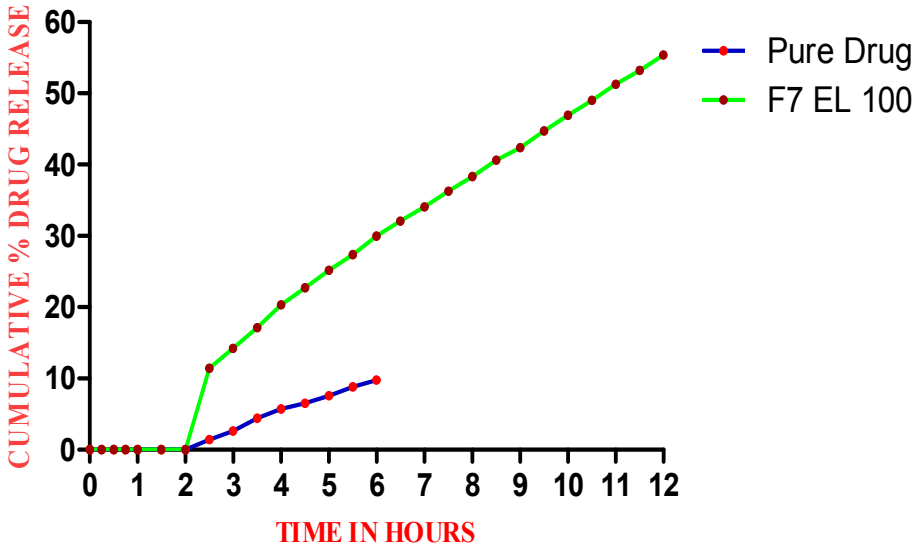

FIGURE 25e: COMPARISON OF IN VITRO DRUG RELEASE PROFILE OF FELODIPINE NANOPARTICLES (F7 EL 100 + 1% PLURONIC F 68) WITH PURE DRUG.

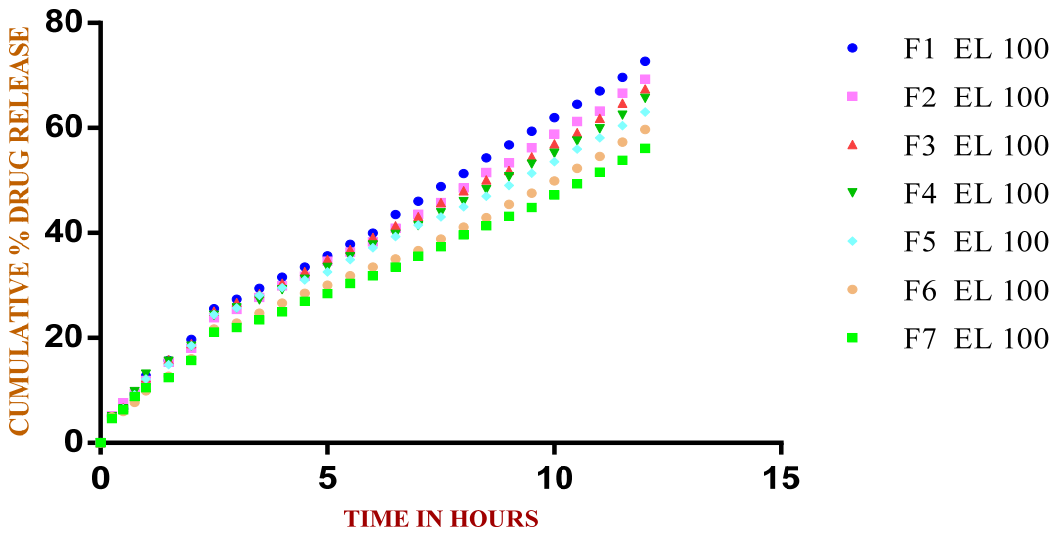

FIGURE 26a: COMPARISON OF *INVITRO* ZERO ORDER RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

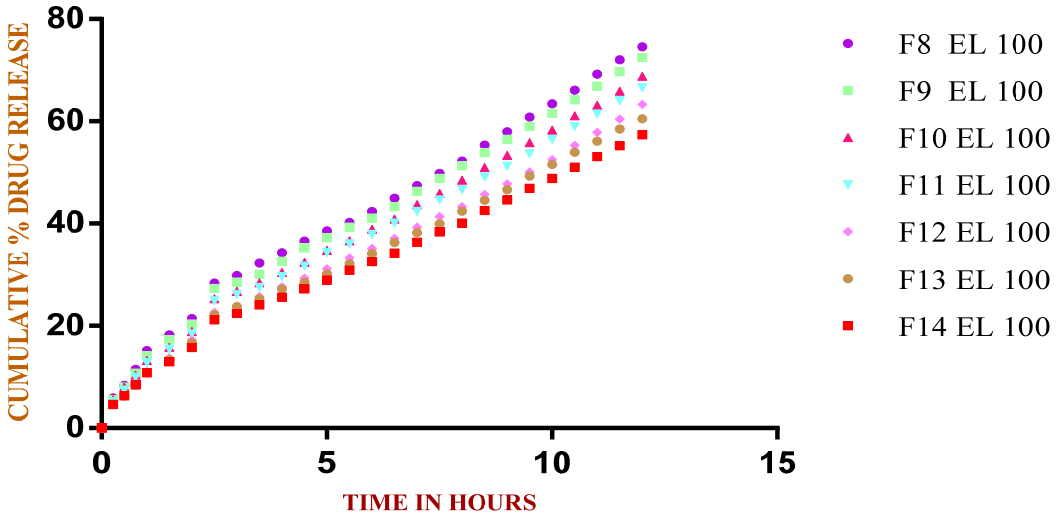

FIGURE 26b: COMPARISON OF *INVITRO* ZERO ORDER RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

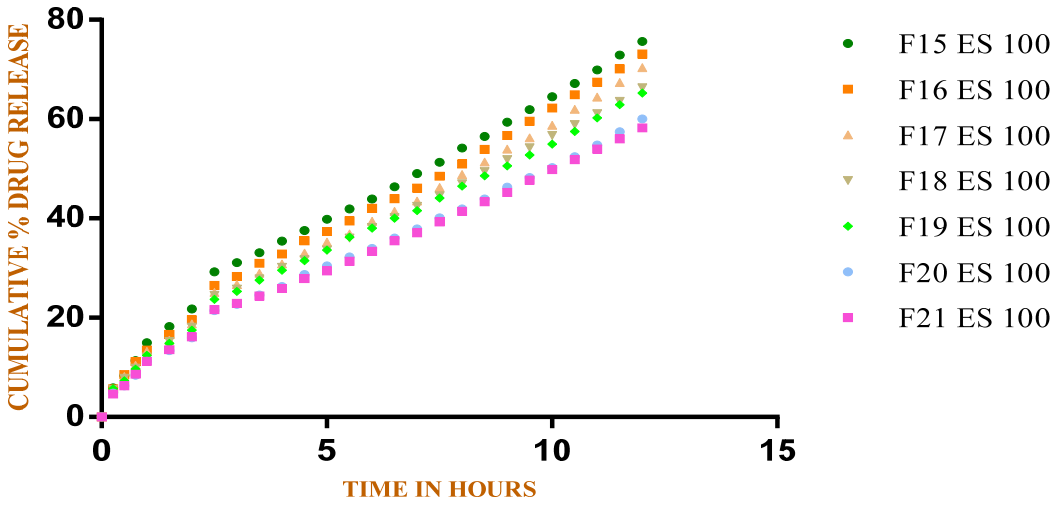

FIGURE 26c: COMPARISON OF *INVITRO* ZERO ORDER RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

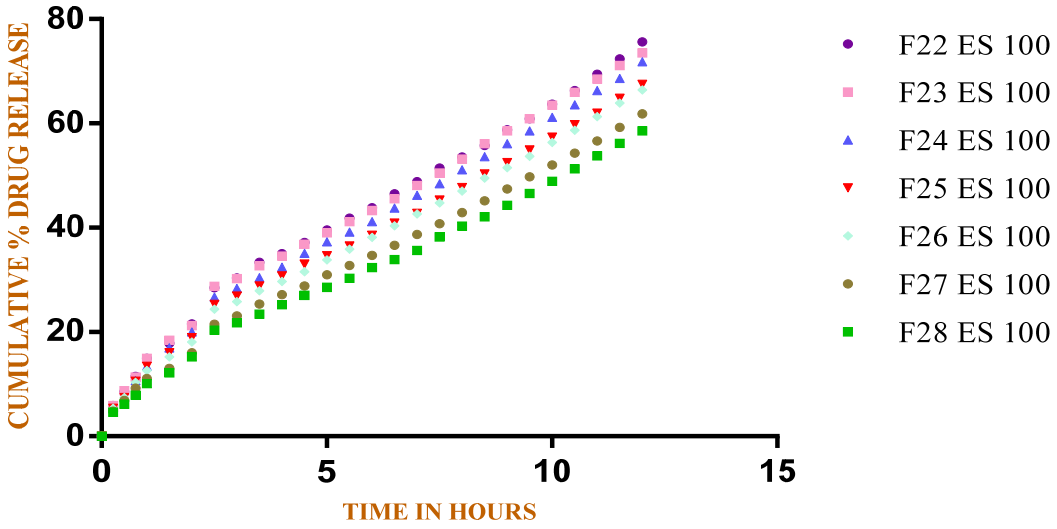

FIGURE 26d: COMPARISON OF *INVITRO* ZERO ORDER RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

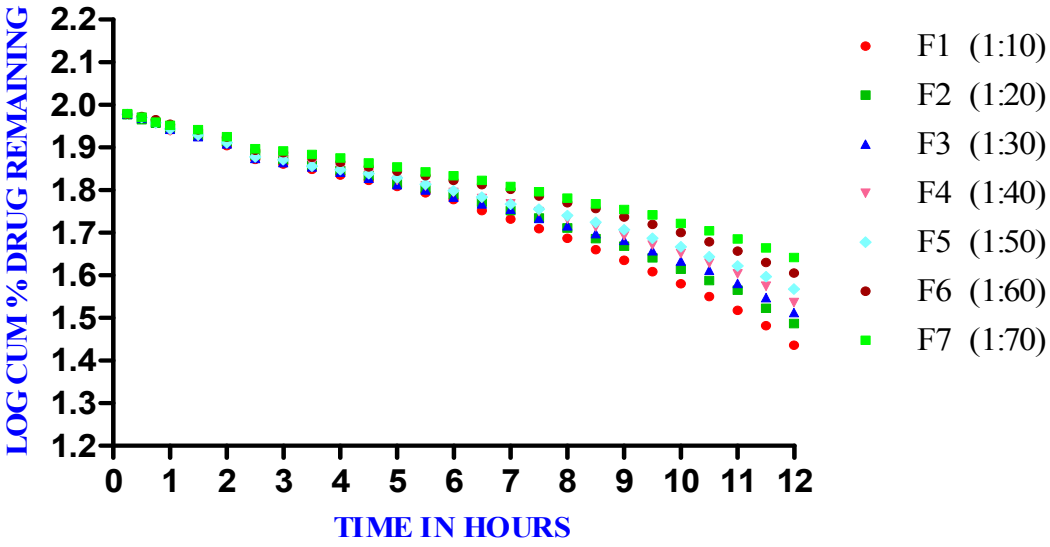

FIGURE 27a: COMPARISON OF *INVITRO* FIRST ORDER RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

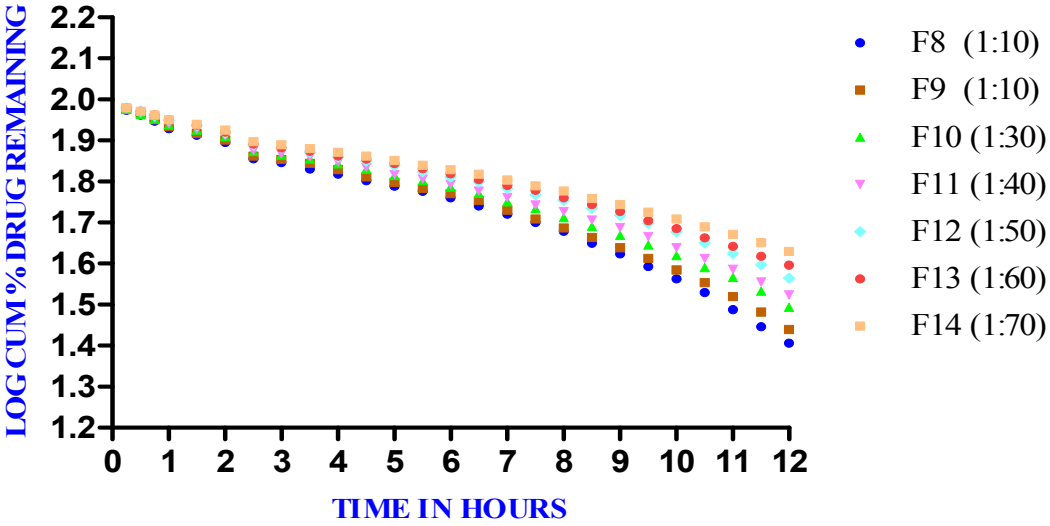

FIGURE 27b: COMPARISON OF *INVITRO* FIRST ORDER RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

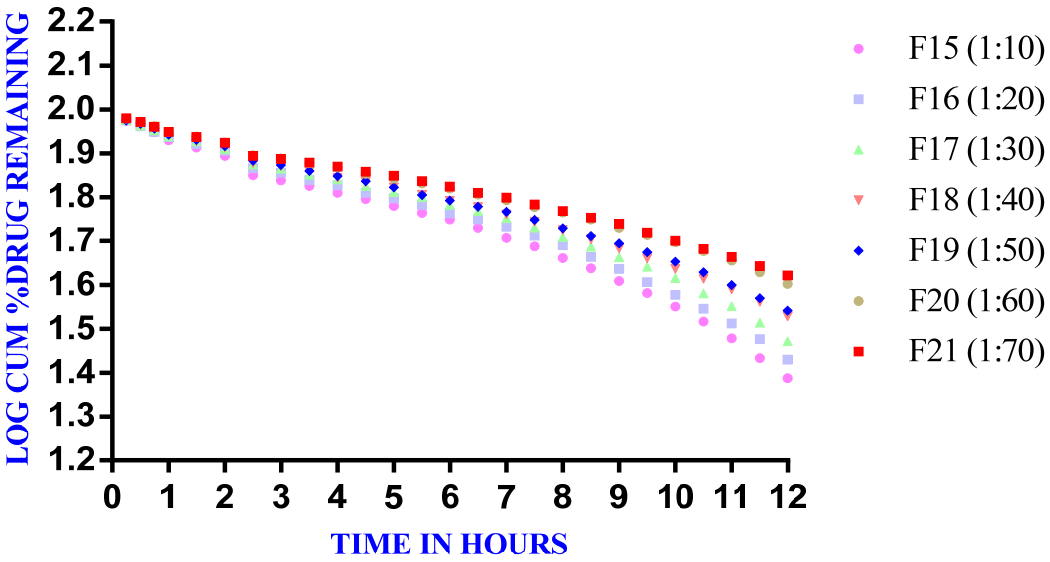

FIGURE 27c: COMPARISON OF *INVITRO* FIRST ORDER RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

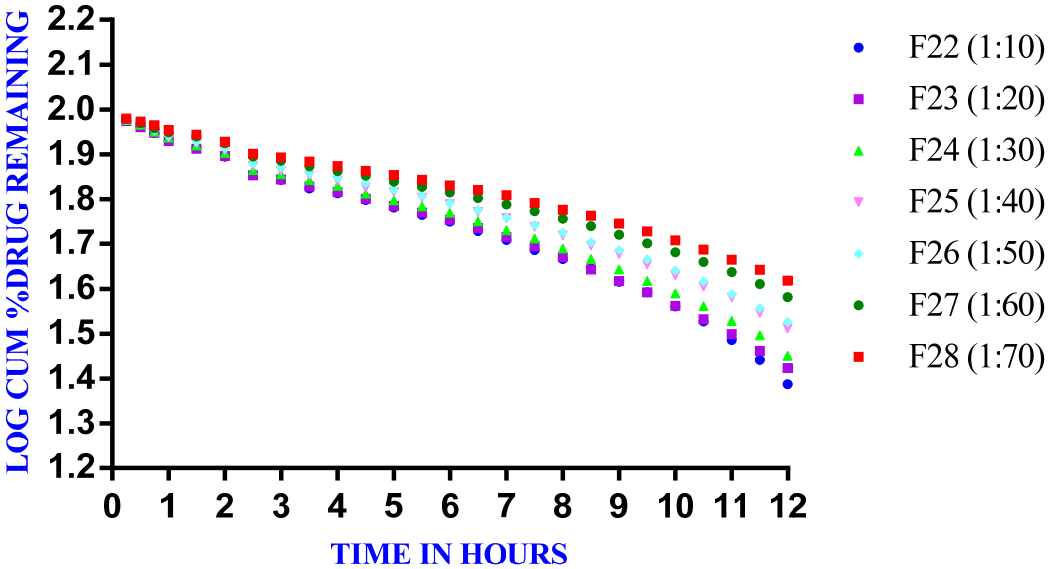

FIGURE 27d: COMPARISON OF *INVITRO* FIRST ORDER RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

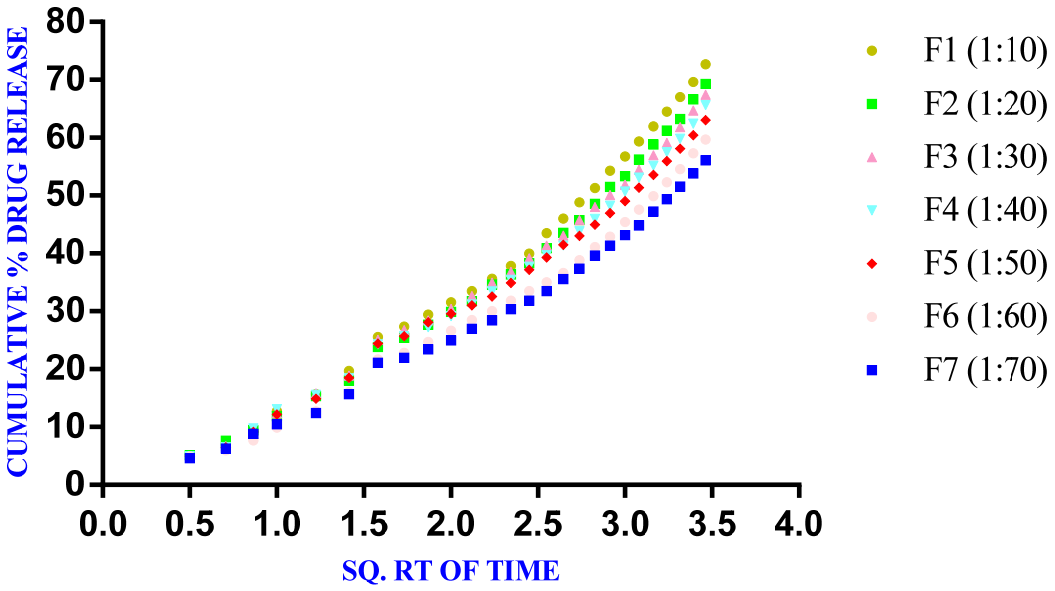

FIGURE 28a: COMPARISON OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

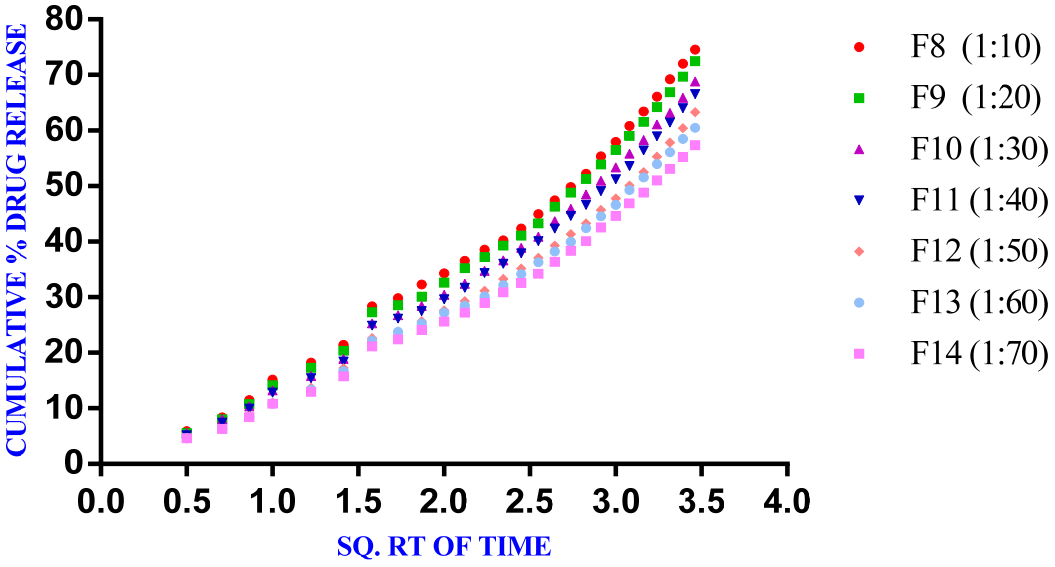

FIGURE 28b: COMPARISON OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

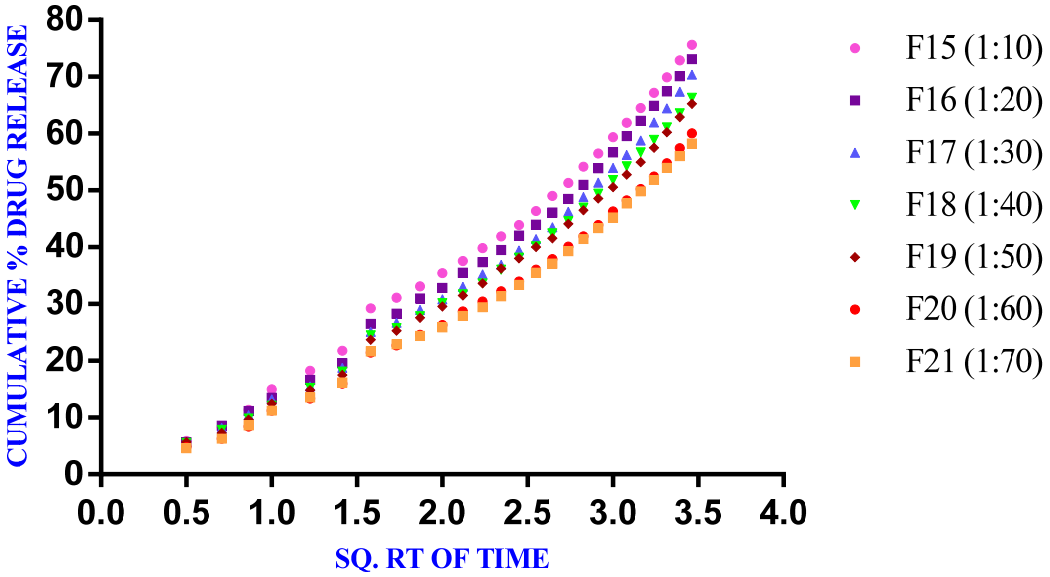

FIGURE 28c: COMPARISON OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

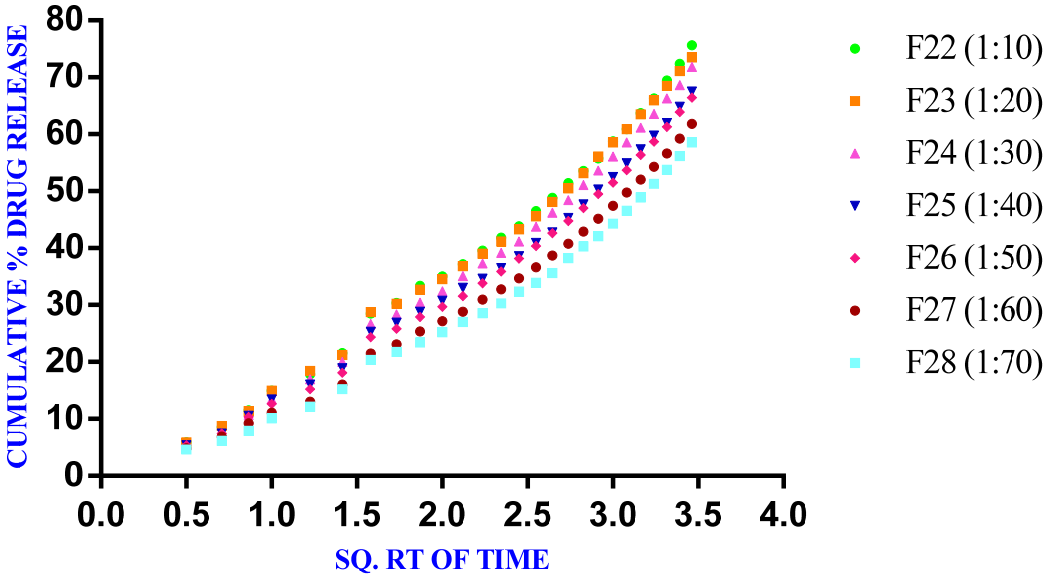

FIGURE 28d: COMPARISON OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

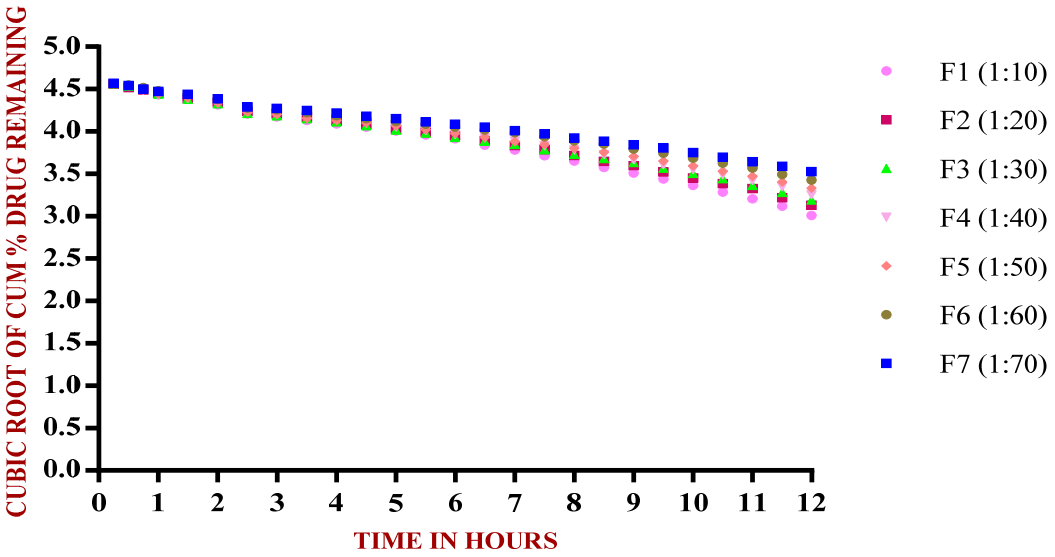

FIGURE 29a: COMPARISON OF *INVITRO* HIXSON CROWELL MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

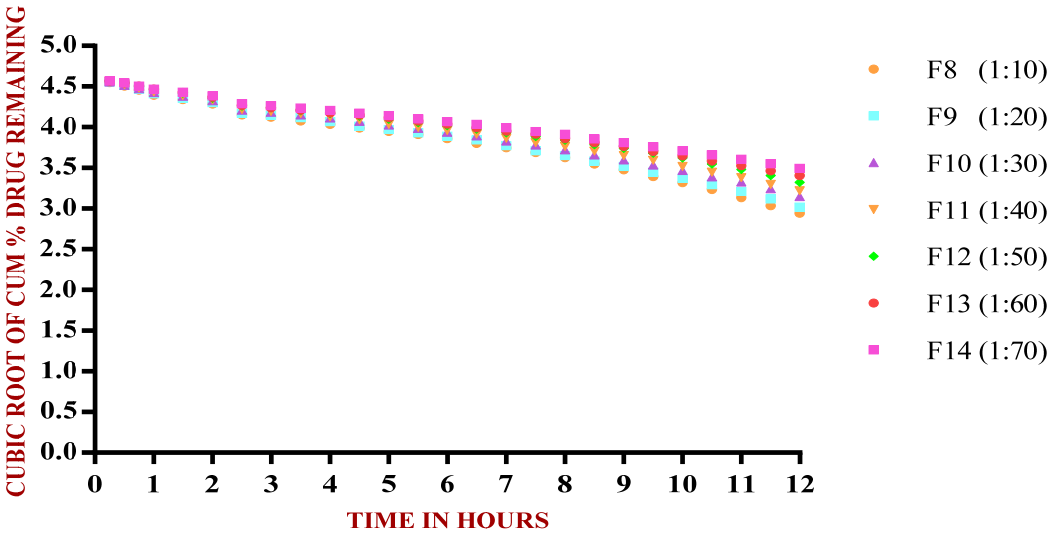

FIGURE 29b: COMPARISON OF *INVITRO* HIXSON CROWELL MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

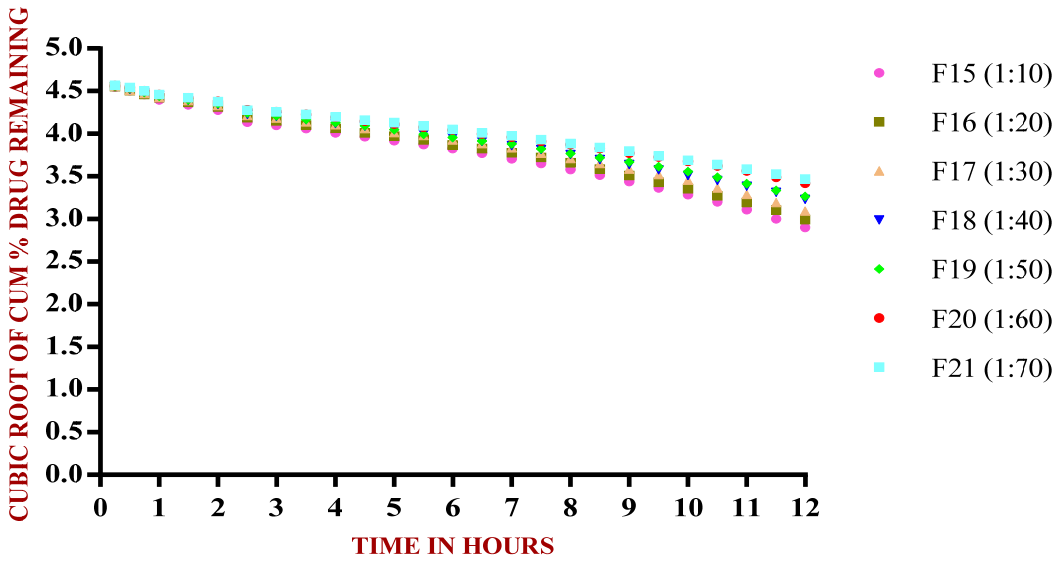

FIGURE 29c: COMPARISON OF *INVITRO* HIXSON CROWELL MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

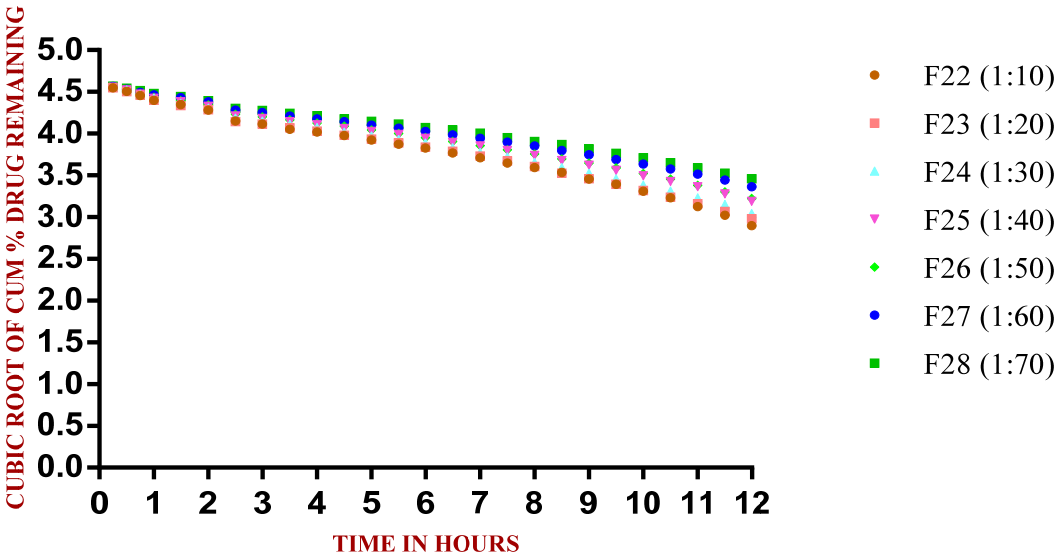

FIGURE 29d: COMPARISON OF *INVITRO* HIXSON CROWELL MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

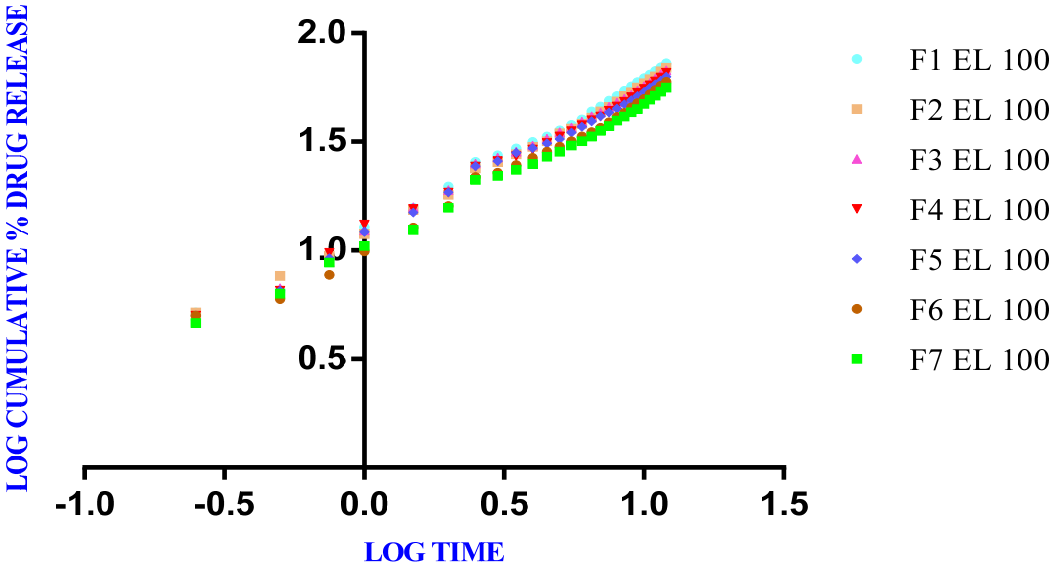

FIGURE 30a: COMPARISON OF *INVITRO* KORSMEYER PEPPAS MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

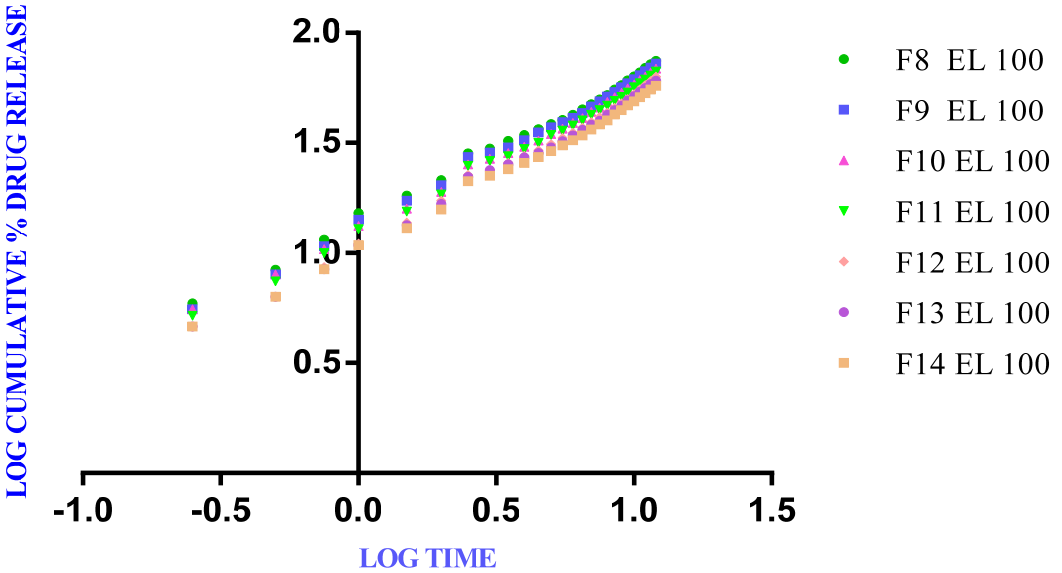

FIGURE 30b: COMPARISON OF *INVITRO* KORSMEYER PEPPAS MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

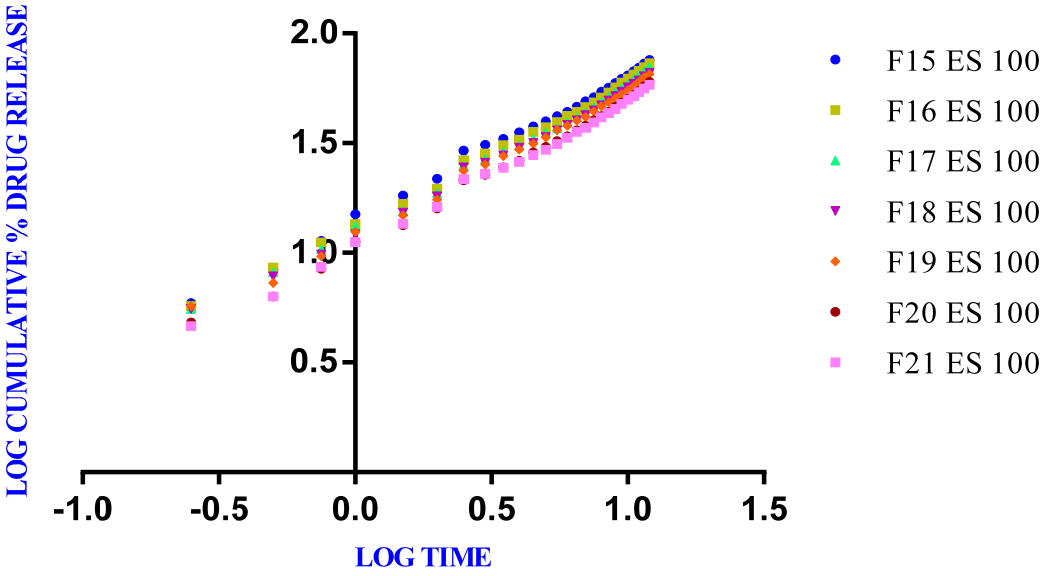

FIGURE 30c: COMPARISON OF *INVITRO* KORSMEYER PEPPAS MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

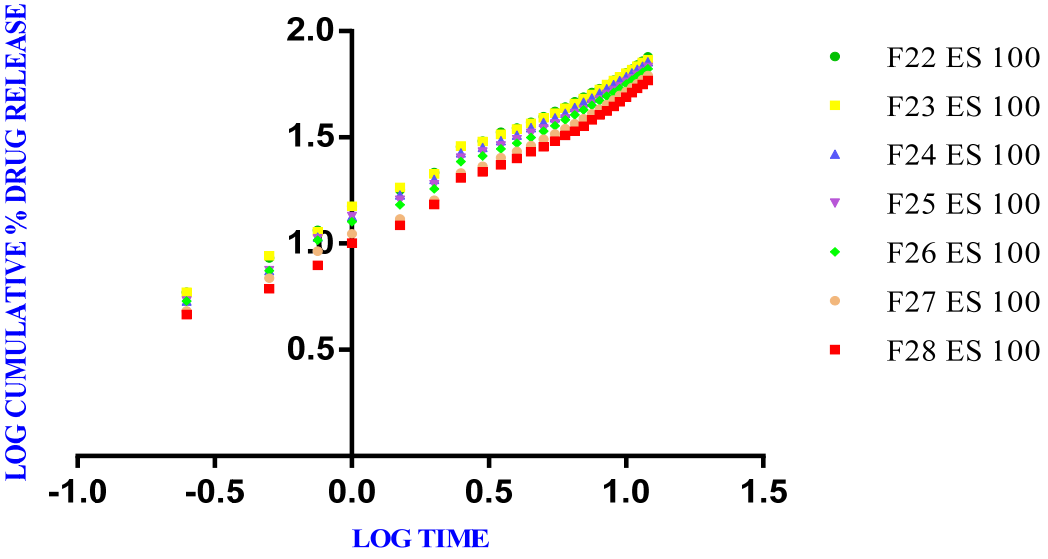

FIGURE 30d: COMPARISON OF *INVITRO* KORSMEYER PEPPAS MODEL RELEASE KINETICS OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

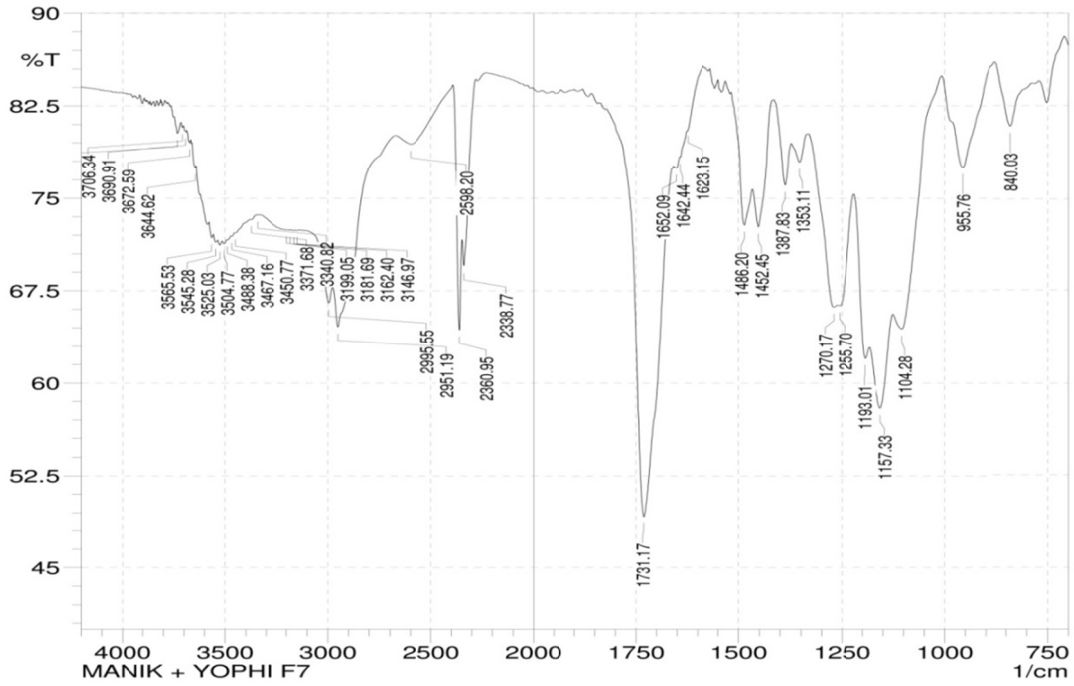

FIGURE 31: INFRARED SPECTAM OF FELODIPINE + EUDRAGIT L 100+ 1%PLURONIC F68

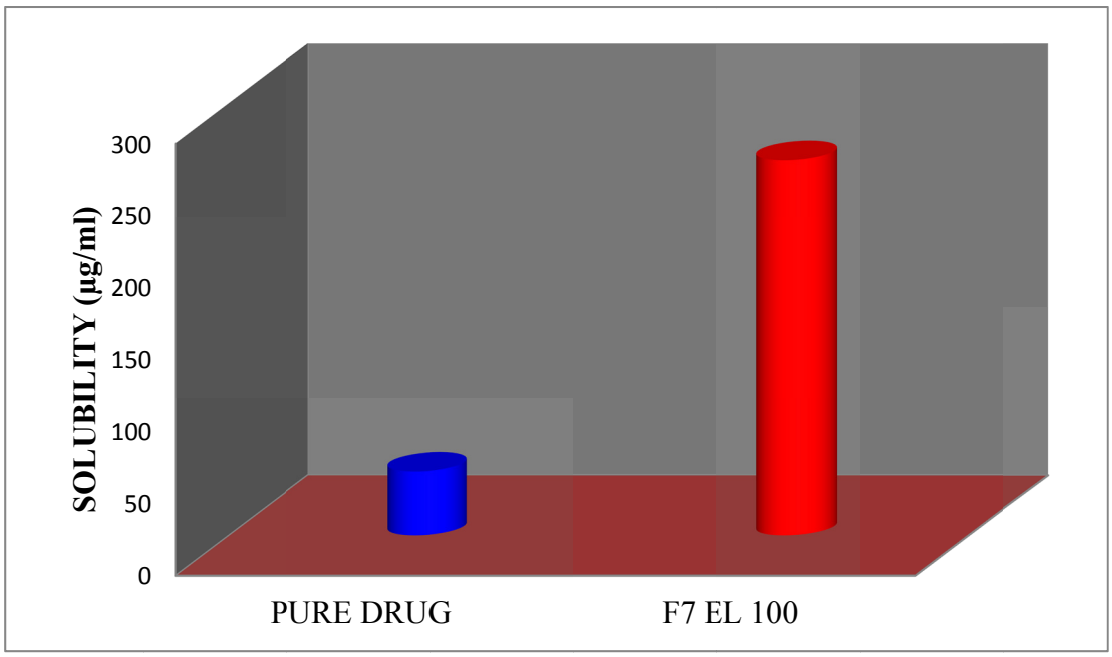

FIGURE 32a: COMPARISON OF SOLUBILITY OF BEST FORMULATION WITH PURE DRUG USING PHOSPHATE BUFFER pH 6.5 with 0.1% SLS

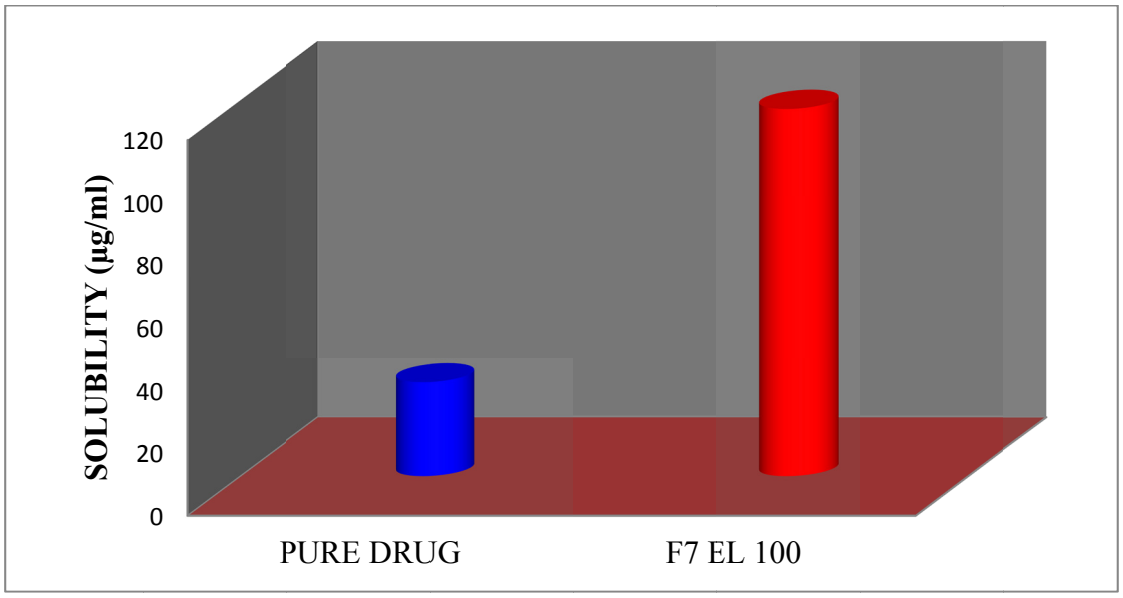

FIGURE 32b: COMPARISON OF SOLUBILITY OF BEST FORMULATION WITH PURE DRUG USING DISTILLED WATER

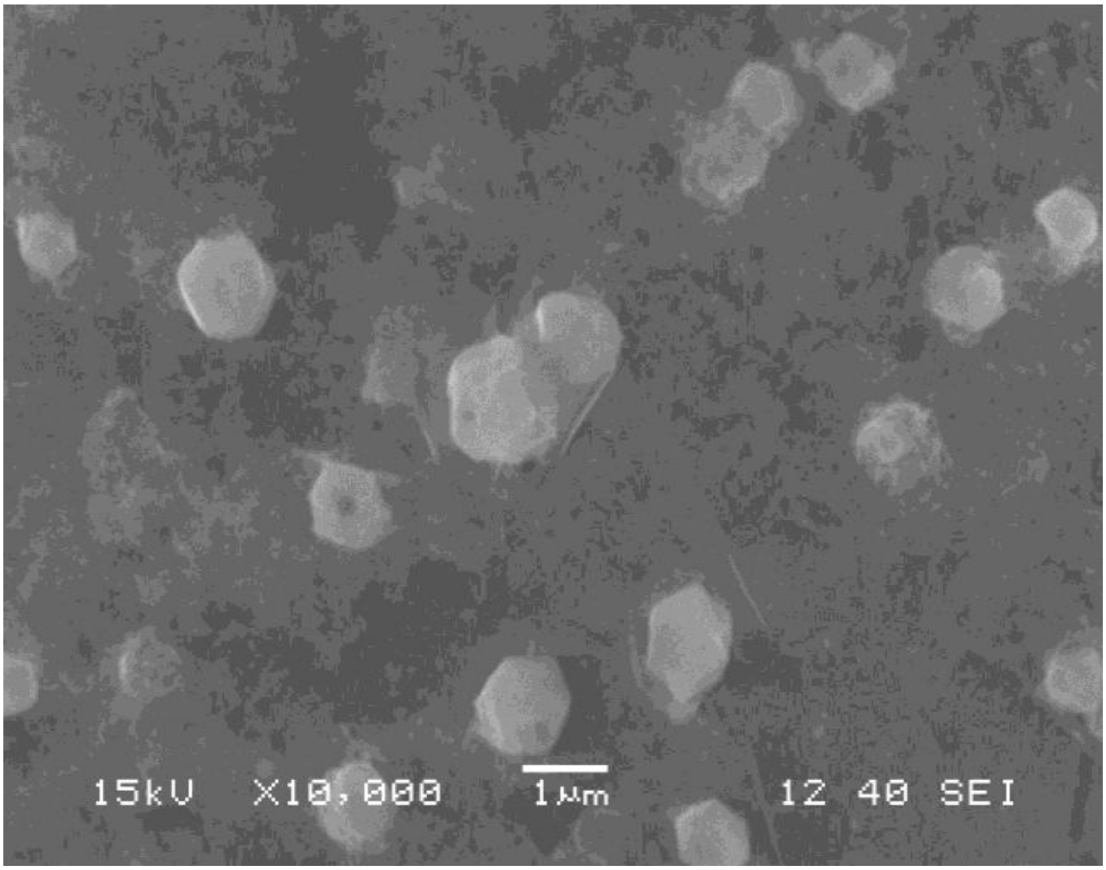

FIGURE 33: SEM IMAGE OF F7 EL 100 + 1% PLURONIC F 68

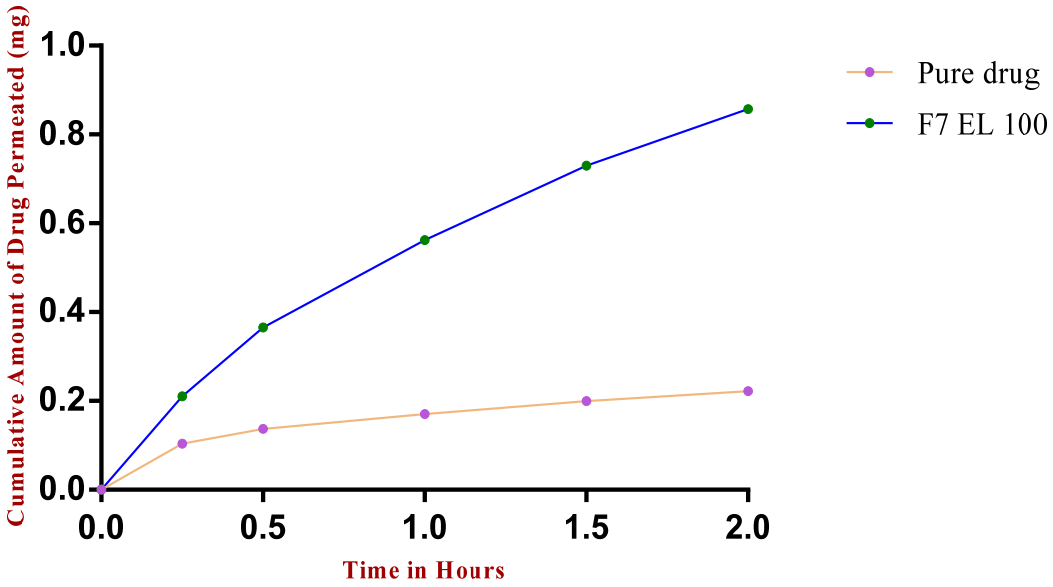

FIGURE 34a: COMPARISON OF CUMULATIVE AMOUNT OF DRUG PERMEATED ACROSS DUODENUM SEGMENT

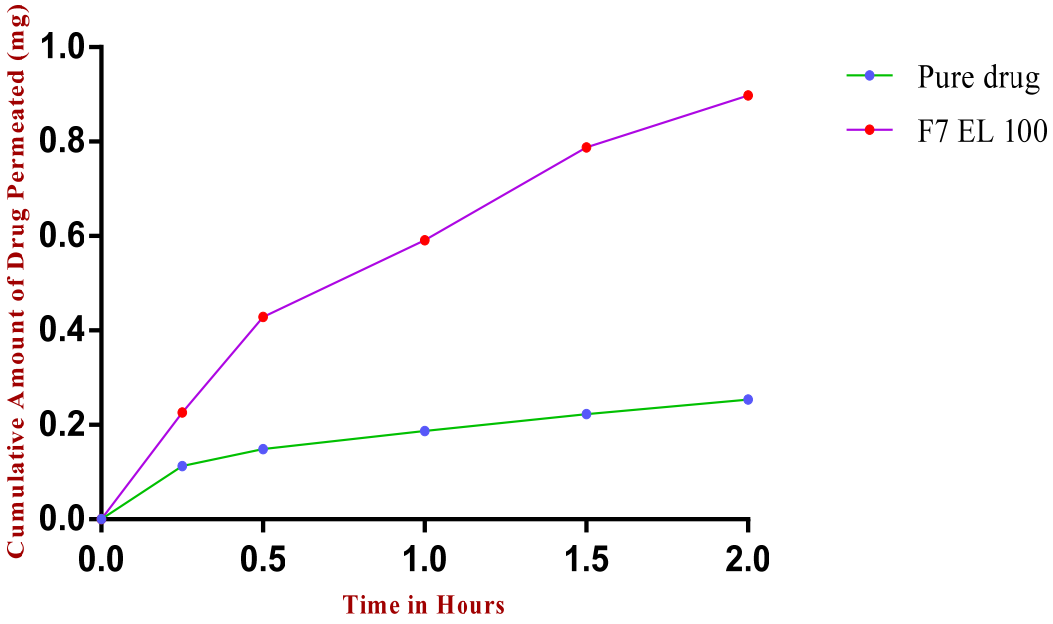

FIGURE 34b: COMPARISON OF CUMULATIVE AMOUNT OF DRUG PERMEATED ACROSS JEJUNUM SEGMENT

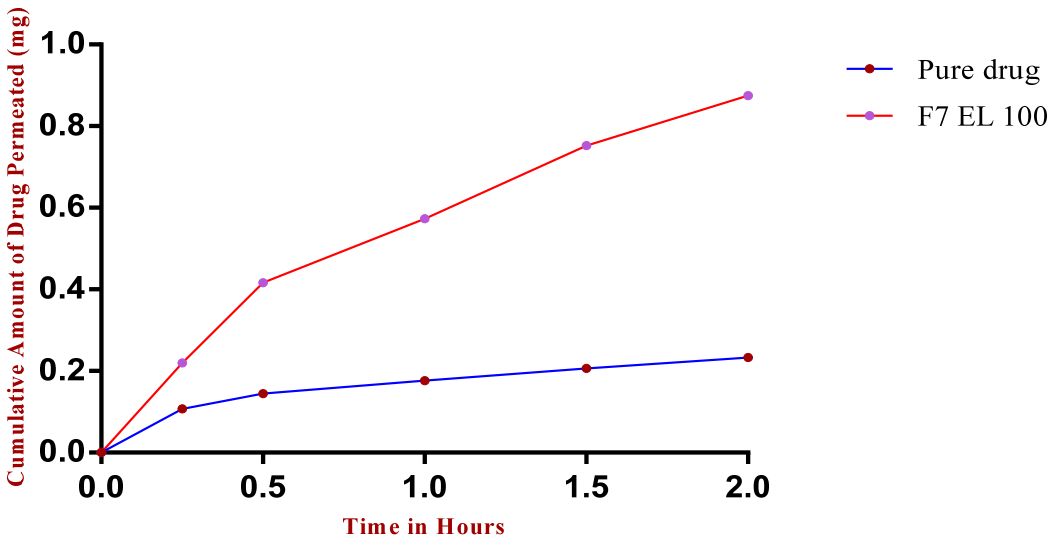

FIGURE34c: COMPARISON OF CUMULATIVE AMOUNT OF DRUG PERMEATED ACROSS ILEUM SEGMENT

CHAPTER XI

SUMMARY AND CONCLUSION

CHAPTERXI

SUMMARY AND CONCLUSION

1. In the present study an attempt was made to develop nanoparticles of felodipine in order to enhance its solubility and dissolution rate by decreasing its particle size to nano level and to sustain its therapeutic activity using Eudragit L and Eudragit S 100.
2. IR spectroscopy studies confirmed that there was no interaction between drug and polymers.
3. The felodipine loaded polymeric nanoparticles were successfully prepared by nanoprecipitation technique using Eudragit L100 and Eudragit S100 as polymers in the presence of stabilizers (Pluronic F 68 and Polyvinyl alcohol).
4. The drug content analysis showed minimum variations suggesting uniform distribution of drug.
5. The entrapment efficiency of all the formulations increased with increasing the concentration of polymers and decreased with increasing concentration of stabilizers.
6. Particle size analyzer used to explore the particle size of felodipine loaded polymeric nanoparticles showed a suitable particle size in the range of 192.4 nm -302.4nm.
7. The polydispersity index of polymeric nanoparticle formulation was less than 0.3, which indicated a relative homogenous dispersion.

8. Zeta potential of felodipine loaded polymeric nanoparticles showed a negative surface charge due to the presence of terminal carboxylic groups in the polymers.
9. The *in vitro* drug release studies displayed a biphasic drug release pattern with a burst release within 2 hours followed by sustained release for 12 hours.
10. *In vitro* drug release kinetics showed sustained release and non fickian diffusion mechanism.
11. On the basis of release data and kinetic analysis F7 showed a good sustained release profile with maximum entrapment efficiency.
12. The solubility of felodipine loaded polymeric nanoparticles increased to ten folds when compared to pure drug solution.
13. SEM analysis of the polymeric nanoparticles showed the spherical shape of the nanoparticles.
14. The results of *ex vivo* intestinal permeability studies showed an increase in the permeation of felodipine loaded polymeric nanoparticles across small intestinal segments when compared to pure drug solution.

Conclusion

Hence, it was concluded that the polymeric nanoparticles prepared by nanoprecipitation is one of the useful method for the successful incorporation of felodipine with high entrapment efficiency. The solubility and *ex vivo* intestinal permeability studies suggested that the nanoparticle formulations can improve the bioavailability of felodipine by improving its solubility. Furthermore, it could be presumed that if the nanometer range of particles were obtained, the bioavailability

might be increased. Hence, we can conclude that polymeric nanoparticles enhanced the bioavailability of poorly water soluble lipophilic drug like felodipine as a drug delivery system.

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ANNEXURE

ANNEXURE

Investigator declaration

- I certify that I have determined that the research proposal herein is not unnecessarily duplicate of previously reported research.
- I certify that all individuals working on this proposal and experimenting on the animals have been trained in animal handling procedures.
- For procedures listed under item 11, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.
- I will obtain approval from the IAEC / CPCSEA before initiating any significant changes in this study.
- Certified that performance of experiment will be initiated only up on review and approval of scientific intent by appropriate expert body (institutional scientific advisory committee / funding agency / other body (to be named)
- Institutional biosafety committee (IBC) certification of review and concurrence will be taken (required for studies utilizing DNA agents of human pathogens)
- I shall maintain all the records as per format (Form D)

A. Manikkavasagan.
Signature

(A.Manikkavasagan)

Name of Investigator

I. A. E. C. CHAIRMAN
INSTITUTIONAL ANIMAL ETHICAL COMMITTEE
K. M. COLLEGE OF PHARMACY
MADURAI-625 107.